Zinc Monotherapy for Young Patients with Presymptomatic Wilson Disease: A Single Center, Retrospective Study

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Research article

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Abstract

Background

Few studies have focused on the treatment failure of zinc monotherapy for presymptomatic Wilson disease (WD) patients. Therefore, we aimed to evaluate the long-term efficacy of zinc monotherapy in presymptomatic patients and to analyze the possible factors that may influence the outcome of this treatment.

Methods

We retrospectively reviewed the medical records of presymptomatic WD patients who received zinc monotherapy from the time of diagnosis. Then, the characteristics of patients who were treated with zinc monotherapy successfully and those who experienced treatment failure were investigated.

Results

Forty presymptomatic WD patients were identified that have received zinc monotherapy as initial treatment, with a median age of 3.83 years at the time of diagnosis. 36 (90%) patients had abnormal alanine transaminase/aspartate transaminase levels at baseline. None of the patients became symptomatic during zinc monotherapy. 28 (70%, Group 1) patients were treated with zinc monotherapy successfully for a median period of 2.8 years. In Group 1, serum aminotransferase levels significantly decreased 6 and 12 months after zinc therapy compared to the baseline levels ($P < 0.05$). 12 (30%, Group 2) patients experienced treatment failure with zinc monotherapy due to uncontrolled serum liver enzyme levels, and D-penicillamine was combined. The baseline 24-hour urine copper levels before treatment were significantly higher in Group 2 compared to that in Group 1 ($P = 0.018$). Comparing the age at onset; ceruloplasmin, serum copper, ALT, and AST levels; and proportions of abdominal ultrasonography abnormality at baseline between Groups 1 and 2 revealed no statistically significant differences.

Conclusions

We found that high initial 24-hour urinary copper levels may lead to treatment failure of zinc monotherapy in presymptomatic WD patients. It might be reasonable to follow up liver function tests more closely during zinc monotherapy and to begin combination treatment with chelators early in patients with high level of 24-hour urinary copper.

Introduction

Wilson disease (WD) is an autosomal recessive disorder caused by mutations in the ATPase copper-transporting beta (*ATP7B)* gene and results in copper accumulation predominantly in the liver, brain, eyes,

and kidneys. The characteristic manifestations of WD are liver disease and cirrhosis, neuropsychiatric disturbances, and the Kayser–Fleischer ring.[1]

Zinc and chelators, such as D-penicillamine (DPA) and trientine, are the main treatments for WD with different clinical phenotypes. Further, it is very important to diagnose WD early because presymptomatic WD patients can remain symptom-free if early, adequate, and persistent treatment is administered.[2] Zinc salts have been successfully used to treat presymptomatic patients; however, so far, there have not been head-to-head studies that have investigated zinc and chelation treatments in this population,[3, 4] and some studies have reported that there are no significant differences between the efficacy of DPA and that of zinc in presymptomatic and neurological patients.[5, 6] Overall, most studies suggest that a favorable outcome could be achieved with zinc monotherapy.[7–14] A recent study in Japan reported on the good efficacy of zinc in maintaining normal transaminase levels in younger presymptomatic patients with WD.[12] However, even in cases in which the patient group was restricted to asymptomatic patients with WD, somen studies have still reported on observing unresponsiveness to zinc therapy or the worsening of symptoms to a certain degree.[1, 14–16] At our center, the poor efficacy of zinc monotherapy in controlling liver enzyme levels has also been observed in a fraction of presymptomatic patients with WD. These conflicting findings indicate that there may be underlying factors that could influence or predict the efficacy of zinc monotherapy in presymptomatic WD patients. Understanding these potential underlying parameters may be useful in formulating more effective or personalized plans for the initial treatment and follow-up in WD patients.

Therefore, we conducted the present retrospective study to investigate the long-term efficacy of zinc monotherapy in presymptomatic patients with WD. We also further analyzed the possible factors that may influence the outcome of zinc monotherapy, with the aim of establishing a possible benchmark to aid in the decision-making process for the initial treatment strategy.

Patients And Methods

We retrospectively reviewed the medical records of all the patients with WD who were referred to the clinic of the First Affiliated Hospital of Sun Yat-Sen University between 2002 and 2012. Patients were eligible for inclusion in the study based on the following criteria: 1) patients who satisfied the diagnostic criteria based on the Leipzig score (≥ 4);[17] 2) patients in whom WD was diagnosed before the development of liver, neurological, or any other manifestations (presymptomatic) with or without the presence of abnormal liver enzymes; 3) patients who were treated with zinc monotherapy from time of diagnosis. The data of clinical and laboratory characteristics, treatment outcomes, changes in treatment regimens, and adverse effect were obtained from medical chart. Then, the characteristics of patients who were treated with zinc monotherapy successfully and those who experience treatment failure were investigated.

For the zinc monotherapy, zinc gluconate was prescribed for all the patients. The initial dosage of elemental zinc for patients under 6 years old was 50 mg/day divided into two separate doses; for those between 6 to 15 years, the dosage was 75 mg/day divided into three doses; and for those who were

16 years and older or whose body weight reached 125 pounds, the dosage was 150 mg/day divided into three doses. Zinc gluconate was administered at least 1 hour before or after a meal. All the patients were asked to avoid food with high copper content. The compliance with the prescribed zinc therapy was evaluated by telephone and personal interviews.

At our center, DPA was prescribed in combination with zinc when treatment failure of zinc monotherapy was determined. Treatment failure with zinc monotherapy was defined as follows. The ongoing and unchanged zinc monotherapy regimen last for at least 6 months before discontinuation. Liver enzyme concentration (alanine transaminase [ALT] or aspartate transaminase [AST]) was more than twice the upper limit of the normal levels for at least two times during follow-up. Conditions apart from WD that lead to elevations in liver enzyme levels were investigated and excluded. The initial dosage of DPA was 20 mg/kg per day divided into two to three doses. The maintenance dose was between 500–750 mg per day divided into two or three doses. DPA and zinc were given separately.

The study was approved by the ethics committee of the First Affiliated Hospital of Sun Yat-Sen University (No. [2014] 23).

Statistical analysis

Continuous variables were expressed as mean ± standard deviation (SD) or median with range in some instances, and categorical variables, as frequency and percentage. Statistical analyses were performed using t-tests or the Mann–Whitney U test as appropriate for quantitative data. The X^2 test or Fisher exact test was used for categorical variables. All the statistical analyses were performed using SPSS V.25 (IBM Corporation, New York, USA). The tests were two-sided, and *P* values of less than 0.05 were considered significant.

Results

Demographics

Forty presymptomatic WD patients were identified that have received zinc monotherapy as initial treatment. The baseline clinical and laboratory characteristics of these 40 presymptomaitc WD patients are presented in Table 1. All patents satisfied the diagnostic criteria based on the Leipzig score. 22 patients had confirmed by genetic test. The mean age at the time of diagnosis was 3.83 years (range, 1.67–18) with 27 (36.7%) males. Six (15%) patients with a median age of 8.5 years were diagnosed during a family screening. 34 (85%) patients with a median age of 4.66 years were found to have unexpected laboratory abnormalities (AST/ALT elevations) when they were subjected to routine blood tests that were conducted either before they begin kindergarten (30 patients) or to test for other diseases (two for upper respiratory tract infection, one for pneumonia, and one for indirect hernia).

Table 1
Clinical and laboratory characteristics at the time of diagnosis in 40 WD patients

Sex (Female/Male)	**13/27**
Age at onset, years, median (range)	3.83 (1.67-18)
Age at diagnosis, years, median (range)	4.79 (1.84-18)
Time onset to diagnosis, months, median(range)	4 (0–55)
Kayser-Fleischer ring, n (%)	2 (5)
Follow-up period, years, median (range)	2.7 (0.3–10.8)
Diagnosis by family screening, n (%)	6 (15)
Diagnosis suspected from unexpected laboratory abnormalities, n (%)	34 (85)
Ceruloplasmin (n = 40), mg/dL, mean ± SD	6.5 ± 2.7
Serum copper (n = 30), mg/L, mean ± SD	0.30 ± 0.15
Urinary copper (n = 40), μg/day, mean ± SD	137.2 ± 81.9
AST (n = 36) U/L mean ± SD	140.5 ± 73.8
ALT (n = 38) U/L mean ± SD	218.5 ± 135.0
Abdominal ultrasonography abnormality n (%)	23 (57.5)

The median follow-up period was 2.7 years (range, 0.3–10.8). At the time of diagnosis, the levels of AST, ALT, ceruloplasmin, and 24-hour urinary copper were 140.5 ± 73.8 U/L, 218.5 ± 135.0 U/L, 6.5 ± 2.7 mg/dL, and 137.2 ± 81.9 μg/day, respectively. Normal serum transferase levels were found in four patients who were diagnosed with WD during a family screening. The Kayser–Fleischer ring was observed in one patient by the slit-lamp examination and in another patient by the naked eye examination.

Analysis of treatment efficacy

Twenty-eight of the 40 patients (70%, Group 1) were treated with zinc monotherapy successfully with the median follow-up of 2.8 years (range: 0.3–27). The other 12 patients (30%, Group 2) experienced treatment failure of zinc monothery and was combined with chelator therapy with DPA (median follow-up: 3.35 years; range: 0.3–10.8). During zinc monotherapy in Group 1 and Group 2, none of them developed any liver-related, neurological, or other symptoms. Zinc and DPA were well tolerated in all patients.

In Group 1, during the entire zinc monotherapy, normal transaminase levels were maintained in three patients (10.71%) who had normal liver enzyme levels when diagnosed by family screenings. 21 patients (75%) exhibited improvements in serum transaminase levels 6 months after zinc treatment. Two patients

(7.14%) exhibited improvements in serum aminotransferase levels within 6 to 12 months after the initiation of zinc therapy. The levels of serum aminotransferases observed 6 and 12 months after zinc therapy were significantly decreased compared to the baseline levels ($P < 0.05$, Fig. 1). Liver enzyme levels continued to decrease during the second year of zinc monotherapy and remained stable during the follow-up period with acceptable fluctuations that mostly remained under 50 U/L between 2 to 6 years. The proportions of patients with normal ALT and AST values were 35%, 50%, and 75% at the 1-, 2- and 6-year timepoints during the treatment, respectively.

In Group 2, 12 patients were prescribed DPA treatment in combination with zinc monotherapy because of the treatment failure. One patient was excluded from the treatment analysis because of incomplete follow-up data. The serum transaminase level measured 6 months after zinc therapy was significantly decreased compared to the baseline level ($P < 0.05$, Fig. 2). However, after 6 months of zinc monotherapy, serum transaminases levels were observed to be poorly controlled. The median duration of zinc monotherapy in Group 2 was 1.9 years (range 0.5–6.4). During zinc monotherapy, the serum transaminase levels in seven (63.6%) patients did not return to normal. The liver enzyme levels have transiently returned to normal in four (36.4%) patients. During the combination therapy of DPA with zinc, serum transaminase levels decreased in six patients within 6 months and normalized in five patients within 15 months.

No statistically significant differences were found between the patients in Group 1 and Group 2 in regarding of age at onset; ceruloplasmin, serum copper, ALT, and AST levels; and the abdominal ultrasonography abnormality ratio at baseline (Table 2). The 24-hour urine copper levels were significantly higher in Group 2 (173 ± 92.9 μg/day) compared to that in Group 1 (114 ± 54.4 μg /day) ($P = 0.018$, Fig. 3). The proportions of the 24-hour urinary copper levels that were over 100 μg/day in Group 2 was significantly higher than that in Group 1 (81.8 vs 42.8%, $P < 0.05$, Table 3).

Table 2
Comparison of baseline data between Group1 and Group 2

	Group 1	Group 2	*P* value
Age at onset, years, median (range)	3.83 (1.67-18)	4.21 (2.17-12)	0.925
Age at diagnosis, years, median (range)	4.83 (1.84-18)	4.71 (2.67-12)	0.743
Ceruloplasmin, mg/dL, mean ± SD	6.4 ± 2.8	6.6 ± 2.4	0.867
Serum copper (mg/L) mean ± SD	0.27 ± 0.15	0.35 ± 0.14	0.208
Urinary copper, μg/day, mean ± SD	114 ± 54.4	173 ± 92.9	0.018
AST U/L mean ± SD	130.8 ± 70.6	163.5 ± 79.7	0.222
ALT U/L mean ± SD	194.5 ± 119.4	277.5 ± 157.9	0.085
Abdominal ultrasonography abnormality n (%)	16 (59.3%)	5 (45.5%)	0.482

Table 3
Comparison of the ratio of 24-hour urinary copper levels that were over 100 μg/day at baseline between Group 1 and Group 2

Group	24-hour urinary copper (n)		P Value
	< 100 μg/24 h	≥ 100 μg/24 h	
Group 1	16	12 (42.8%)	0.037
Group 2	2	9 (81.8%)	

Discussion

Zinc therapy is reported to be a good option as a first-line treatment for asymptomatic WD patients. However, unresponsiveness to zinc therapy or the worsening of symptoms has been observed to occur in some case series. Nonetheless, there are no reported or known influencing factors that affect the efficacy of zinc monotherapy in presymptomatic WD patients.

In this study, we found that all of the included patients remained asymptomatic during zinc monotherapy, indicating that zinc monotherapy was effective in preventing the occurrence of liver-related, neurological, or other symptoms in presymptomatic WD patients. This is consistent with previous reports and current guidelines.

The patients in Group 1 responded well to zinc monotherapy and were kept on this therapy for a median duration of 2.8 years. Serum transaminase levels improved in 83.1% of them during the first year after the initiation of zinc therapy and continued to decrease during the second year. Liver enzyme levels remained stable during the follow-up period with acceptable levels of fluctuation. This is in line with the findings of previous studies involving asymptomatic WD patients.[11–13, 18,19] These reported good responses to zinc treatment were considered to base on the role zinc plays in inhibiting intestinal copper absorption by inducing the enterocytes to synthesize metallothionein and in the depletion of stored copper.[11, 20–22]

However, the same regimen of zinc monotherapy resulted in treatment failure in Group 2. We found that baseline 24-hour urine copper level was significantly higher in Group 2 compared to that in Group 1 (P = 0.018). Patients in Group 2 also showed a higher proportion of the 24-hour urine copper level that were over 100 μg/day. High 24-hour urinary copper levels are indicative of a high copper burden in the liver cells and other organs. Thus, the slow copper removal facilitated by zinc perhaps could not match the speed of copper accumulation, which leads to a failure in achieving a negative copper balance. As a result, the copper toxicity could not be alleviated, and liver enzymes would exhibit an unsatisfactory decrease or uncontrolled increase. This finding might be the underlying explanation for the poor response to zinc treatment observed in Group 2 and in other studies.[1, 14–16] Lamireau,[1] Mishra,[14] Weiss,[23] and Linn[8] have reported on the poor efficacy of zinc monotherapy in presymptomatic or symptomatic WD patients. However, these studies did not analyze and compare the baseline 24-hour urinary copper values

between zinc responders and non-responders. Of course, poor compliance with the treatment regimen and an inadequate dosage of zinc may also result in treatment failure. Ranucci has reported on a good long-term efficacy of zinc monotherapy in 86% of children with WD, and only two patients (14%) were non-responders to zinc.[13] It was found that these two patients complied poorly with the treatment regimen, as inadequate levels of serum and urinary zinc were uncovered.

Non-compliance with anti-copper therapy was reported in both asymptomatic and symptomatic patients, and it has a definite negative effect on clinical outcome.[2,24] Unfortunately, in our study, serum and urine zinc levels were not measured, and compliance was only assessed by telephone or personal interviews, which was not an objective and adequate technique. Consequently, we could not determine whether the zinc dosage was appropriate for the patients. It is reported that patients treated with zinc should have urinary zinc levels of > 2000 μg/day and serum zinc levels of > 150 mch/dl.[25] These zinc levels would be observed if patients were to adhere to the zinc treatment regimen and if the zinc were properly absorbed. Therefore, poor compliance should be taken into consideration as a possible reason for the treatment failure of zinc monotherapy in Group 2. Weiss and Linn uncovered the early elevation trend of the ALT and gamma-glutamyltransferase (γ-GT) levels in zinc nonresponders.[8, 23] For the patients in Group 2, we prescribed combination therapy of DPA and zin, considering that DPA and zinc have different mechanisms. The combination therapy works toward both blocking copper uptake and eliminating excess copper, which might result in better efficacy. After combining with DPA, the serum transaminase levels decreased in six patients within six months and normalized in five patients within 15 months. There are also some studies that have reported on the simultaneous use of chelators and zinc. However, further studies are required to determine whether the efficacy of the combination therapy is greater than that of chelator therapy alone.

In developed countries, the most commonly used zinc salt is zinc acetate. However, the current guidelines do not provide a clear recommendation for the appropriate zinc formulation. It is thought that the type of zinc salt that is used does not make a difference with respect to efficacy. We used zinc gluconate in this study, and it exhibited good efficacy and tolerability. A recent study also found that WD can be treated effectively using zinc acetate or alternative zinc preparations, including zinc gluconate.[26] These findings support the view that when treating a patient with zinc, the most important factor is an adequate dosage of elemental zinc, but not the type of zinc salt chosen.

Till now, there are still no clearly defined parameters that could predict treatment efficacy of zinc for WD. For the presymptomatic WD patients in our study, the serum liver enzyme level might be the only laboratory sign that exhibits changes in the early stage of WD. The double upper limit of normal transaminase level was frequently used to evaluate the treatment response of zinc therapy in the studies.[6, 7, 12–13, 23] Iorio et al. reported on the phenomenon of persistent hypertransaminasemia despite appropriate treatment with penicillamine or zinc.[27] They found that 11 (50%) out of 22 patients treated with zinc and 29 (33%) out of 87 patients treated with DPA had persistent hypertransaminasemia. One interesting finding was that none of these patients with persistent hypertransaminasemia exhibited a

worsening of liver disease or developed other WD-related symptoms, which drew the attention to the possibility that expensive time-consuming diagnostic evaluations and ineffectual changes in therapy could be avoided. However, in the zinc group of Iorio's study,[27] five patients were switched to DPA, and 60% of them exhibited the normalization of ALT levels, which indicated that DPA might be necessary for a fraction of these WD patients. However, the underlying mechanism was not clear yet. The high 24-hour urinary copper levels at baseline that we found in our study may give rise to a possible explanation.

Our study indicates the good efficacy of zinc therapy in most presymptomatic WD patients. It underlines the view that a close observation of liver function is essential during zinc monotherapy in presymptomatic WD patients, especially in those with high 24-hour urinary copper levels. If liver enzyme levels are not well controlled within 2 years, it is reasonable to consider that zinc therapy should be combined with or switched to chelator therapy in order to alleviate copper toxicity as soon as possible. However, for presymptomatic WD patients with high 24-hour urinary copper levels, especially for those with levels above 100 μg/day, a narrower observation time window of perhaps less than 1 year should be considered for the cases in whom liver function is not well controlled.

Our study has some limitations. First, there was bias that compliance was only evaluated by telephone and personal interview without objective parameters, such as serum and urine zinc levels. Second, 24-hour urinary copper level during follow-up was incomplete due to retrospective nature of the study, which was unable to further analysis in regarding of the treatment compliance and outcomes. Third, the liver enzyme levels were not followed-up closely enough to ascertain the best observation period to assess the efficacy of zinc monotherapy. Forth, statistical analysis was limited by the small sample size in our study, which is justified by the nature of the disease.

Conclusions

In conclusion, we found that high initial 24-hour urinary copper levels, especially those above 100 μg/day, may lead to the treatment failure of zinc monotherapy in presymptomatic WD patients. This finding indicates the need for a closer follow-up of liver function and a shorter observation period during zinc monotherapy. It may be reasonable to consider a combination treatment with chelators early in order to achieve progressive elimination of copper.

Abbreviations

WD: Wilson disease; DPA: D-penicillamine; ALT: Alanine transaminase; AST: Aspartate transaminase; SD: standard deviation

Declarations

Ethics approval and consent to participate

The study was approved by the ethics committee of the First Affiliated Hospital of Sun Yat-Sen University (No. [2014] 23). Written consent was waived as the study collected data from reviewing medical records of standard care.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

XH L, XL L, and HM H conceived and designed the study; HM H, DB C, JX L, L F and JW Z collected data; HM H, YM X and XH L analyzed the data; HM H drafted the manuscript. XH L, XL Land YM X substantively revised the manuscript. All authors have read the and approved the manuscript.

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Figures

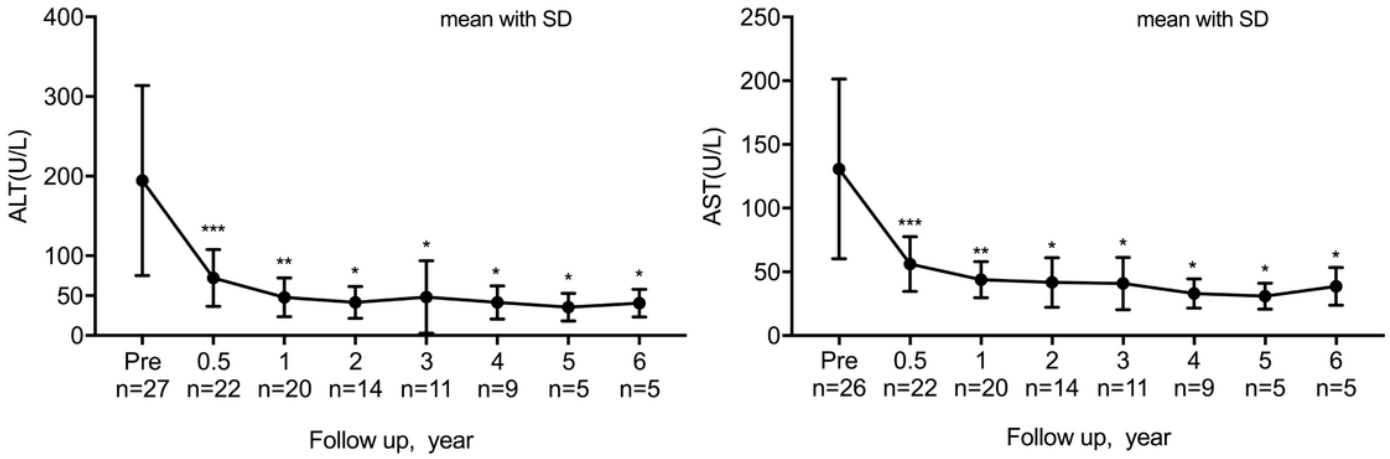

Figure 1

Serum liver aminotransferases levels before and after zinc monotherapy in Group 1 *** P < 0.001 versus Pre, ** P < 0.01 versus 0.5-year, * P > 0.05 versus the previous follow up

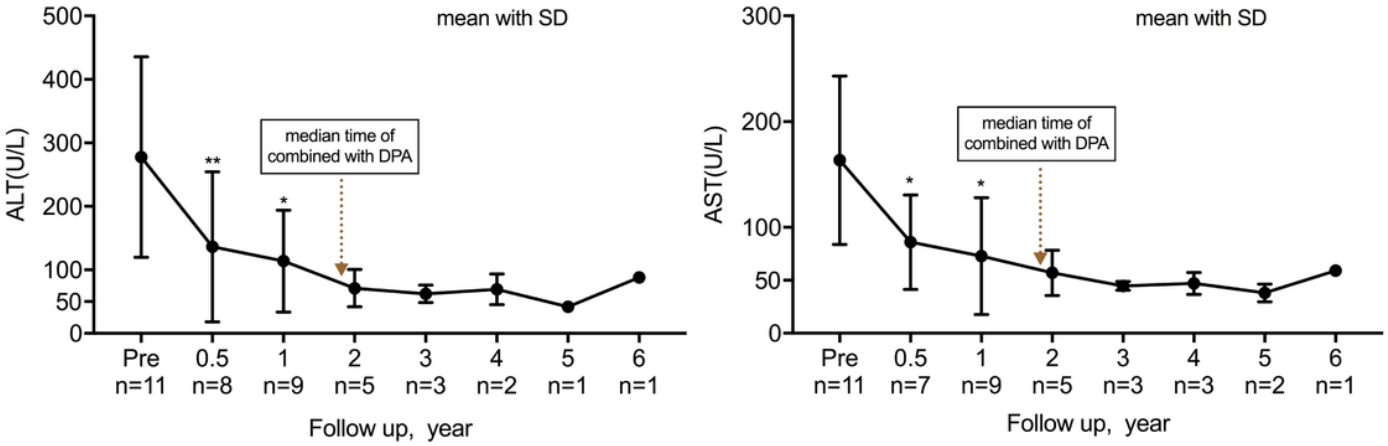

Figure 2

Serum liver aminotransferases levels before and after zinc monotherapy in Group 2 ** P < 0.01 versus pre, * P < 0.05 versus the previous follow up

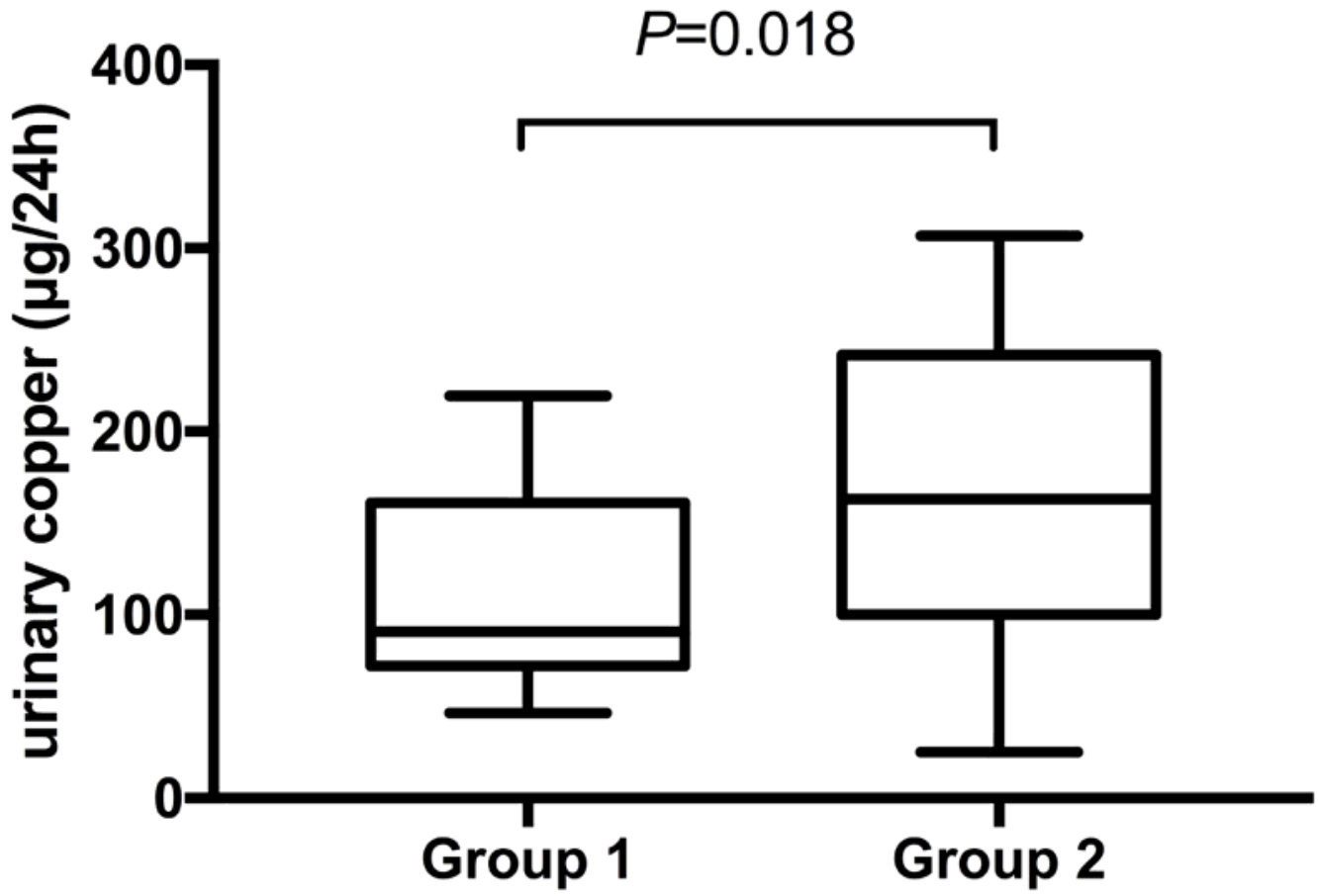

Figure 3

Comparison of 24-hour urinary copper level at baseline between Group 1 and Group2