Title: **J-shaped relationship between serum zinc levels and the severity of hepatic necro-inflammation in patients with MAFLD**

Short Title: Serum zinc levels and MAFLD severity

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List of Abbreviations

MAFLD, metabolic dysfunction-associated fatty liver disease; NAFLD, non-alcoholic fatty liver disease; HN, hepatic necro-inflammation; MHN, mild hepatic necro-inflammation; SHN, severe hepatic necro-inflammation; FIB-4, fibrosis-4; BMI, body mass index; HOMA-IR, homeostasis model assessment of insulin resistance; CI, confidence interval; IQR, inter-quartile ranges; LRT test, likelihood ratio test; HCC, hepatocellular carcinoma; IR, insulin resistance; PTP1B, protein tyrosine phosphatase 1b; ZAG, zinc-α2-glycoprotein.

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ABSTRACT

Background and Aims: Zinc is an essential trace element that plays an important role in maintaining health, and affecting gene expression, signal transduction and regulation of apoptosis. It is uncertain whether serum zinc levels are altered in patients with metabolic dysfunction-associated fatty liver disease (MAFLD). We aimed to investigate the association between serum zinc levels and the severity of hepatic necro-inflammation (HN) in patients with MAFLD.

Methods and Results: Liver disease severity was graded histologically using the NAFLD activity score. HN was defined as the sum of ballooning and lobular inflammation. We used a smooth function regression model to analyze the relationship between serum zinc levels and HN. A total of 561 (76.5% men) patients with biopsy-confirmed MAFLD were enrolled. They had a mean age of 41.3 years, and a mean serum zinc level of 17.0 ± 4.1 μmol/L. Compared to those with mild hepatic necro-inflammation (MHN, grades 0-2; n=286), patients with severe hepatic necro-inflammation (SHN, grades 3-5; n=275) had lower serum zinc concentrations (16.3±4.2 vs. 17.6±4.0 μmol/L; $p<0.001$). However, a threshold saturation effect analysis showed that there was an inflection in serum zinc levels at 24 μmol/L. After adjustment for potential confounders, serum zinc levels <24 μmol/L were inversely associated with SHN (adjusted-odds ratio 0.88, 95%CI 0.83-0.93; $p<0.001$), whereas serum zinc levels >24 μmol/L were positively associated with SHN (adjusted-odds ratio 1.42, 95%CI: 1.03-1.97; $p=0.035$).

Conclusions: There is a J-shaped relationship between serum zinc levels and the

severity of hepatic necro-inflammation in patients with biopsy-proven MAFLD.

Keywords: MAFLD, serum zinc, liver biopsy, hepatic necro-inflammation

Introduction

Fatty liver disease related to metabolic dysfunction affects up to nearly 30% of the world's adult population [1]. It has been proposed that non-alcoholic fatty liver disease (NAFLD) should be redefined and re-classified as metabolic dysfunction-associated fatty liver disease (MAFLD), because this newly proposed definition better reflects the pathogenic role of metabolic dysfunction associated with fatty liver disease [2, 3]. There is growing evidence that histological characteristics in both NAFLD and MAFLD may similarly identify patients at higher risk of liver disease progression, and the prevalence of NAFLD and MAFLD is essentially comparable using vibration-controlled transient elastography [4, 5]. Furthermore, it is known that this common metabolic liver disease is not only associated with metabolic syndrome (MetS) and types 2 diabetes (T2D) [6], but also with other important extra-hepatic complications, such as cardiovascular disease and chronic kidney disease [7, 8].

Zinc is an essential trace element, which has an important role in the human body to maintain health [9]. Over 300 enzymes depend on zinc for their functions, and zinc also plays an important role in regulating the immune system [10]. Some studies have shown that reductions of enzyme activities caused by zinc deficiency can be restored with zinc supplementation [11, 12]. Zinc deficiency also induces endoplasmic reticulum stress, leading to hepatic steatosis and apoptosis [13]. However, a recent small meta-analysis of 8 studies showed that serum zinc levels were lower in NAFLD patients than in healthy controls, but the study did not find any significant differences in dietary zinc intake between these two groups of subjects, thus suggesting that there might be altered absorption or utilization of zinc in NAFLD [14]. Additionally, zinc transporters play a role in biofilms, and their dysfunction may lead to disorders of zinc homeostasis and metabolic disease [15]. Previous small studies have suggested that lower serum zinc levels may be associated with greater hepatic lobular inflammation and fibrosis [16, 17], as well as higher values of fibrosis (FIB)-4 score [18] in subjects with NAFLD. Another recent study in patients with MAFLD (where liver fibrosis was assessed by vibration-controlled transient elastography) did not show any significant difference in dietary zinc intake between patients with advanced fibrosis and those without advanced fibrosis [19]. However, it remains uncertain whether serum zinc levels are associated with the full range of liver histological changes in patients with MAFLD.

Therefore, the aim of this large cross-sectional study was to investigate the association between serum zinc levels and the severity of liver histological changes in a well-phenotyped cohort of Chinese individuals with biopsy-proven MAFLD.

Materials and Methods

Study population

We recruited consecutive Chinese adults with biopsy-confirmed MAFLD from the PERSONS cohort (2017-2020). The definition of MAFLD was based on the recent diagnostic criteria proposed by an international expert panel [1]. Our study cohort included patients from a previously published study as well as additional subjects [20, 21]. The exclusion criteria for our study were as follows: (1) liver fat content < 5% on liver histology; (2) hepatic steatosis on histology, but not coexisting MAFLD-associated metabolic abnormalities (as specified below); (3) refusal of liver biopsy; and (4) data missing for serum zinc levels. The protocol for the study was approved by the local ethics committee of our hospital. Each participant gave informed written consent.

Clinical and laboratory data

Clinical and laboratory data were collected in all participants within 24 hours of liver biopsy examination. Body mass index (BMI) was calculated as body weight (kg)/ height square (m^2). Waist circumference was measured at the midpoint between the lower margin of the last palpable rib and the top of the iliac crest. Homeostasis model assessment of insulin resistance (HOMA-IR) score was calculated as [insulin (pmol/L)/ 6.965] x glucose (mmol/L)/ 22.5. All blood samples were taken in the morning, after an overnight fast, including serum liver enzymes (alanine aminotransferase, aspartate aminotransferase and gamma-glutamyltranspeptidase), total bilirubin, albumin, hemoglobin A1c (HbA1c), glucose, insulin, blood urea nitrogen, creatinine, high sensitivity C-reactive protein (hs-CRP), as well as lipid profile (total cholesterol, triglycerides, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol).

Serum zinc measurement

Zinc ions were detected by the colorimetric method of Olympus (AU5831), Japan [22]. The linear correlation coefficient of performance of the serum zinc test kit was (r) ≥ 0.995, and the inter-batch coefficient of variation (CV) of serum zinc levels was 6% in the normal range (11.6-25.5 μmol/L).

Definitions

MAFLD was diagnosed according to recently proposed criteria [2]. These diagnostic criteria dictate that hepatic steatosis (as detected by biopsy, imaging techniques, or non-invasive biomarkers), together with either a diagnosis of T2D, or overweight/obesity (BMI ≥ 23 kg/m^2, in Asian people), or the presence of metabolic dysregulation (as defined by the presence of at least two of the seven metabolic risk factors), are required to establish a diagnosis of MAFLD. Hypertension was diagnosed as systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 85 mmHg and/or use of any anti-hypertensive drugs [23]. Diagnosis of diabetes was based on a prior medical history of diabetes, fasting glucose ≥126 mg/dL (≥7.0 mmol/L), hemoglobin A1c ≥ 6.5% (≥48 mmol/mol), and/or use of any anti-hyperglycemic agents [24].

Liver histology

All participants underwent ultrasonic-guided percutaneous core liver biopsies (detailed procedure referenced in our previous publication [25]), and each liver specimen was evaluated by an experienced liver pathologist and scored according to the NAFLD Activity Score (NAS) system. The NAS is the sum of three histological components, which include hepatic steatosis (0-3), ballooning (0-2), and lobular inflammation (0-3) [26]. Liver fibrosis is graded from 0 to 4 according to Brunt's histological score [27]. Hepatic necro-inflammation (HN) was defined as the sum of ballooning and lobular inflammation, which were classified as mild hepatic necro-inflammation (MHN, grades 0-2) and severe hepatic necro-inflammation (SHN, grades 3-5) [28]. Hepatic steatosis was classified as mild (grades 1) and severe (grades 2-3) [28]. Liver fibrosis was histologically classified as mild (stages 0-1) and severe (stages 2-4) [29].

Statistical analysis

Continuous variables were expressed as means ± standard deviation or medians with interquartile ranges (IQRs), and categorical variables as proportions (%). We used the unpaired Student's *t*-test to analyze continuous data and the chi-square test to analyze categorical variables, respectively. Smooth curve analysis and saturation threshold analysis were used to test the association between serum zinc levels and the presence of severe hepatic necro-inflammation. All statistical analyses were performed using Empower (R) (www.empowerstats.com, X&Y solutions Boston, MA, USA) and R (http://www.R-project.org). Statistical significance was defined as p-values < 0.05.

Results

Baseline characteristics of MAFLD patients

As detailed in **Figure 1**, 805 individuals from the PERSONS cohort were initially identified who were not consuming any zinc supplements. The following subjects were excluded from analysis: 82 cases with no hepatic steatosis (liver fat content < 5%) on histology; 73 cases with hepatic steatosis but no MAFLD-related metabolic abnormalities; 32 patients who refused liver biopsy; and 30 cases with missing data for serum zinc levels. After these exclusions, a total of 561 adults with biopsy-confirmed MAFLD were included in the final analysis. In accord to the newly proposed MAFLD definition, patients with MAFLD in this study included individuals with fatty liver associated with metabolic abnormalities (as detailed in the Methods section above), irrespective of the coexistence of significant alcohol consumption or hepatitis B or C virus infection.

Table 1 shows the demographic, anthropometric, and biochemical variables in all participants stratified by severity of HN. The proportions of single aetiology MAFLD between the MHN and the SHN were 82.5% vs. 89.5%, respectively (p=0.018). Among the dual aetiology MAFLD patients, the rates of excessive alcohol consumption and viral hepatitis B or C in the MHN and SHN were 11.2% vs. 5.5% and 7.0% vs. 5.8%, respectively. In total, 429 (76.5%) were men, with a mean age of 41.3 years, and a mean serum zinc level of 17.0 ± 4.1 μmol/L. The levels of serum zinc ranged between 7 to 31 μmol/L, and 6.8% of patients exceeded 24 μmol/L. Compared to those with MHN, patients with SHN had significantly higher circulating levels of hs-CRP, liver enzymes, total bilirubin, total cholesterol, triglycerides, fasting insulin, and HOMA-IR score. In addition, patients with SHN also had significantly lower levels of serum creatinine and zinc concentrations. Finally, patients with SHN had a higher proportion of severe hepatic steatosis than those with MHN, whereas the stages of fibrosis did not differ significantly between the two patient groups.

We did not find any associations between serum zinc levels and the degree of hepatic steatosis (P= 0.875) or the stage of liver fibrosis (P= 0.590) (data not shown).

Smooth curve and saturation threshold effect analysis

We analyzed whether there was a non-linear association between serum zinc levels and the presence of SHN using a smoothing fitting curve. After adjustment for age, sex, BMI, serum liver enzymes, glucose, triglycerides, total cholesterol and HOMA-IR score, we found that there was a J-shaped association between serum zinc levels and the risk of having SHN, as shown both in **Figure 2** and in **Table 2**.

In particular, the curve analysis of the threshold saturation effect revealed that there was an inflection point for serum zinc levels at 24 μmol/L (**Table 2**). Specifically, we found that serum zinc levels were inversely associated with SHN below the threshold (serum zinc level < 24 μmol/L) of the inflection point (adjusted-OR 0.88, 95% CI 0.83-0.93; p<0.001) after adjustment for the aforementioned potential confounders. Conversely, above this inflection point (zinc > 24 μmol/L), there was a significant positive association between serum zinc levels and SHN (adjusted-OR 1.42, 95% CI:

1.02-1.97; p=0.035).

Discussion

The main and novel results of our cross-sectional study are that there was a J-shaped association between serum zinc levels and the presence of SHN in Chinese adults with biopsy-confirmed MAFLD. The threshold saturation effect analysis showed that there was a threshold effect when serum zinc level was 24 μmol/L. In particular, there was a negative association between serum zinc levels and SHN when serum zinc level was lower than the threshold (24 μmol/L), and a positive association when serum zinc level was equal to or greater than this threshold (where the risk of having SHN was increased by up to nearly 40%). Zinc is found in superoxide dismutase (SOD) and this enzyme plays a key role as an antioxidant in the inflammatory process in hepatocytes [30]. When serum zinc is deficient, it has been suggested that there is decreased antioxidant activity, increased lipid peroxidation and hepatocellular damage in chronic hepatitis [31]. Previous studies investigating the relationship between serum zinc levels and liver disease have shown a linear relationship between the exposure (zinc) and the outcome (liver disease) [17]. Our study is the first to show a non-linear relationship between serum zinc levels and liver histology in MAFLD. We suggest that when serum zinc levels exceed a certain threshold, only then are serum zinc levels positively associated with SHN. Both zinc deficiency and zinc excess also play an important role in the immune system and this may be involved in promoting the development of inflammatory disease [32]. We failed to find any significant association between serum zinc levels and the stages of liver fibrosis. The histological severity of liver fibrosis in patients in our database was mild and we aim to explore the relationship between severe liver fibrosis and serum zinc concentrations in the future. Therefore, in the present study we were only able to study the relationship between serum zinc levels and the histological severity of necroinflammation.

Normal zinc transport plays an important role in both endoplasmic reticulum stress and reduced disease risk [13]. Some metabolic disorders have been found to be associated with lower levels of serum zinc, including T2D [33, 34], and insulin resistance (IR) [35]. One of the possible reasons why this association was observed is that zinc deficiency may lead to increased inflammatory biomarkers due to changes in zinc transporters, as well as reduced suppression of protein tyrosine phosphatase 1b (PTP1B), which has been linked to greater IR and hepatic steatosis [36, 37]. Zinc-α2-glycoprotein (ZAG) is an adipokine which was recently associated with obesity and obesity-related metabolic diseases. Adipokine-α2-glycoprotein appears to play a role in lipid metabolism and adipokine-α2-glycoprotein has previously been shown to be significantly reduced in NAFLD [38]. However, whether serum ZAG concentrations play a role in predicting NAFLD amongst patients with metabolic syndrome is uncertain [39]. Altered serum zinc levels may also be related to the development of hepatocellular carcinoma (HCC) [40], and zinc deficiency has been used to estimate the survival rates of patients with early HCC [41].

The major strengths of our study are the relatively large sample size and the use of liver biopsy for diagnosing and staging MAFLD. In addition, we excluded patients with coexisting conditions known to affect serum zinc levels (e.g., patients with end-stage renal disease or chronic diarrhea, those with intestinal malabsorption, or those consuming any zinc supplements). One of the most important limitations of our single-center study is its cross-sectional design that does not allow us to establish causality for the observed association between serum zinc levels and liver disease severity. Moreover, there were few patients with different stages of liver fibrosis to be able to test the relationships between serum zinc levels and advanced liver fibrosis. Finally, the Chinese ethnicity of our participants may preclude the generalizability of these findings to different ethnic groups.

In conclusion, the results of our study show for the first time that there is a significant J-shaped association between serum zinc levels and the presence of severe hepatic necro-inflammation in Chinese individuals with biopsy-proven MAFLD. Further studies are required to corroborate these findings in other ethnic populations and to better understand the mechanistic links between serum zinc levels and hepatic necro-inflammation in MAFLD.

References

[1] Eslam M, Sanyal AJ, George J. MAFLD: A Consensus-Driven Proposed Nomenclature for Metabolic Associated Fatty Liver Disease. Gastroenterology.

2020;158:1999-2014.e1.

[2] Eslam M, Newsome PN, Sarin SK, Anstee QM, Targher G, Romero-Gomez M, et al. A new definition for metabolic dysfunction-associated fatty liver disease: An international expert consensus statement. J Hepatol. 2020;73:202-9.

[3] Lin S, Huang J, Wang M, Kumar R, Liu Y, Liu S, et al. Comparison of MAFLD and NAFLD diagnostic criteria in real world. Liver Int. 2020;40:2082-9.

[4] Zheng KI, Sun D-Q, Jin Y, Zhu P-W, Zheng M-H. Clinical utility of the MAFLD definition. Journal of hepatology. 2020.

[5] Ciardullo S, Perseghin G. Prevalence of NAFLD, MAFLD and associated advanced fibrosis in the contemporary United States population. Liver Int. 2021;41:1290-3.

[6] Marchesini G, Bugianesi E, Forlani G, Cerrelli F, Lenzi M, Manini R, et al. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. Hepatology. 2003;37:917-23.

[7] Guerreiro GTS, Longo L, Fonseca MA, de Souza VEG, Alvares-da-Silva MR. Does the risk of cardiovascular events differ between biopsy-proven NAFLD and MAFLD? Hepatol Int. 2021;15:380-91.

[8] Sun DQ, Jin Y, Wang TY, Zheng KI, Rios RS, Zhang HY, et al. MAFLD and risk of CKD. Metabolism. 2021;115:154433.

[9] Hara T, Takeda T-A, Takagishi T, Fukue K, Kambe T, Fukada T. Physiological roles of zinc transporters: molecular and genetic importance in zinc homeostasis. J Physiol Sci. 2017;67:283-301.

[10] Sanna A, Firinu D, Zavattari P, Valera P. Zinc Status and Autoimmunity: A Systematic Review and Meta-Analysis. Nutrients. 2018;10.

[11] Olechnowicz J, Tinkov A, Skalny A, Suliburska J. Zinc status is associated with inflammation, oxidative stress, lipid, and glucose metabolism. J Physiol Sci. 2018;68:19-31.

[12] King JC. Zinc: an essential but elusive nutrient. Am J Clin Nutr. 2011;94:679S-84S.

[13] Kim M-H, Aydemir TB, Kim J, Cousins RJ. Hepatic ZIP14-mediated zinc transport is required for adaptation to endoplasmic reticulum stress. Proceedings of the National Academy of Sciences of the United States of America. 2017;114:E5805-E14.

[14] Yazihan N. Serum zinc level and dietary zinc intake status in non-alcoholic fatty liver disease: A meta-analysis and systematic review. Hepatology Forum. 2020.

[15] Kambe T, Tsuji T, Hashimoto A, Itsumura N. The Physiological, Biochemical, and Molecular Roles of Zinc Transporters in Zinc Homeostasis and Metabolism. Physiol Rev. 2015;95:749-84.

[16] Kosari F, Jamali R, Ramim T, Mosavi Jahan Abad E. The Correlation between Serum Zinc Level and Liver Histology in Non-Alcoholic Steatohepatitis. Iran J Pathol. 2019;14:17-25.

[17] Ito T, Ishigami M, Ishizu Y, Kuzuya T, Honda T, Ishikawa T, et al. Correlation of serum zinc levels with pathological and laboratory findings in patients with nonalcoholic fatty liver disease. Eur J Gastroenterol Hepatol. 2020;32:748-53.

[18] Kim MC, Lee JI, Kim JH, Kim HJ, Cho YK, Jeon WK, et al. Serum zinc level and hepatic fibrosis in patients with nonalcoholic fatty liver disease. PloS one. 2020;15:e0240195.

[19] Guveli H, Kenger EB, Ozlu T, Kaya E, Yilmaz Y. Macro- and micronutrients in metabolic (dysfunction) associated fatty liver disease: association between advanced fibrosis and high dietary intake of cholesterol/saturated fatty acids. 9000.

[20] Zhou YJ, Zheng KI, Wang XB, Sun QF, Pan KH, Wang TY, et al. Metabolic-associated fatty liver disease is associated with severity of COVID-19. Liver international : official journal of the International Association for the Study of the Liver. 2020;40:2160-3.

[21] Sun D-Q, Zheng KI, Xu G, Ma H-L, Zhang H-Y, Pan X-Y, et al. PNPLA3 rs738409 is associated with renal glomerular and tubular injury in NAFLD patients with persistently normal ALT levels. Liver international : official journal of the International Association for the Study of the Liver. 2020;40:107-19.

[22] Johnsen O, Eliasson R. Evaluation of a commercially available kit for the colorimetric determination of zinc in human seminal plasma. International journal of andrology. 1987;10:435-40.

[23] Alberti KG, Zimmet P, Shaw J. Metabolic syndrome--a new world-wide definition. A Consensus Statement from the International Diabetes Federation. Diabet Med. 2006;23:469-80.

[24] Sabanayagam C, Khoo EY, Lye WK, Ikram MK, Lamoureux EL, Cheng CY, et al. Diagnosis of diabetes mellitus using HbA1c in Asians: relationship between

HbA1c and retinopathy in a multiethnic Asian population. J Clin Endocrinol Metab. 2015;100:689-96.

[25] Sun DQ, Zheng KI, Xu G, Ma HL, Zhang HY, Pan XY, et al. PNPLA3 rs738409 is associated with renal glomerular and tubular injury in NAFLD patients with persistently normal ALT levels. Liver Int. 2020;40:107-19.

[26] Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology. 2005;41:1313-21.

[27] Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. Am J Gastroenterol. 1999;94:2467-74.

[28] Liu WY, Eslam M, Zheng KI, Ma HL, Rios RS, Lv MZ, et al. Associations of Hydroxysteroid 17-beta Dehydrogenase 13 Variants with Liver Histology in Chinese Patients with Metabolic-associated Fatty Liver Disease. J Clin Transl Hepatol. 2021;9:194-202.

[29] Zhou YJ, Ye FZ, Li YY, Pan XY, Chen YX, Wu XX, et al. Individualized risk prediction of significant fibrosis in non-alcoholic fatty liver disease using a novel nomogram. United European Gastroenterol J. 2019;7:1124-34.

[30] Stanković MN, Mladenović D, Ninković M, Ethuričić I, Sobajić S, Jorgačević B, et al. The effects of α-lipoic acid on liver oxidative stress and free fatty acid composition in methionine-choline deficient diet-induced NAFLD. J Med Food. 2014;17:254-61.

[31] Kolachi NF, Kazi TG, Afridi HI, Kazi N, Kandhro GA, Shah AQ, et al. Distribution of copper, iron, and zinc in biological samples (scalp hair, serum, blood, and urine) of Pakistani viral hepatitis (A-E) patients and controls. Biol Trace Elem Res. 2011;143:116-30.

[32] Maywald M, Wessels I, Rink L. Zinc Signals and Immunity. Int J Mol Sci. 2017;18.

[33] Fernández-Cao JC, Warthon-Medina M, V HM, Arija V, Doepking C, Serra-Majem L, et al. Zinc Intake and Status and Risk of Type 2 Diabetes Mellitus: A Systematic Review and Meta-Analysis. Nutrients. 2019;11.

[34] Shan Z, Bao W, Zhang Y, Rong Y, Wang X, Jin Y, et al. Interactions between zinc transporter-8 gene (SLC30A8) and plasma zinc concentrations for impaired glucose regulation and type 2 diabetes. Diabetes. 2014;63:1796-803.

[35] Bjørklund G, Dadar M, Pivina L, Doşa MD, Semenova Y, Aaseth J. The Role of Zinc and Copper in Insulin Resistance and Diabetes Mellitus. Curr Med Chem. 2020;27:6643-57.

[36] Kim M-H, Aydemir TB, Kim J, Cousins RJ. Hepatic ZIP14-mediated zinc transport is required for adaptation to endoplasmic reticulum stress. Proceedings of the National Academy of Sciences. 2017;114:E5805-E14.

[37] Vatsalya V, Kong M, Cave MC, Liu N, Schwandt ML, George DT, et al. Association of serum zinc with markers of liver injury in very heavy drinking alcohol-dependent patients. J Nutr Biochem. 2018;59:49-55.

[38] Qi XY, Li JY, Wang YD, Zeng YW, Liao ZZ, Ran L, et al. Association of serum

zinc-α2-glycoprotein with non-alcoholic fatty liver disease. Chin Med J (Engl). 2020;133:1882-3.

[39] Yilmaz Y, Yonal O, Eren F, Kurt R, Celikel CA, Ozdogan O, et al. Serum zinc-α2-glycoprotein concentrations in patients with non-alcoholic fatty liver disease. Clin Chem Lab Med. 2011;49:93-7.

[40] Fang AP, Chen PY, Wang XY, Liu ZY, Zhang DM, Luo Y, et al. Serum copper and zinc levels at diagnosis and hepatocellular carcinoma survival in the Guangdong Liver Cancer Cohort. Int J Cancer. 2019;144:2823-32.

[41] Hiraoka A, Nagamatsu K, Izumoto H, Adachi T, Yoshino T, Tsuruta M, et al. Zinc deficiency as an independent prognostic factor for patients with early hepatocellular carcinoma due to hepatitis virus. Hepatol Res. 2020;50:92-100.

TABLES LEGEND

Table 1. Baseline characteristics of patients with biopsy-proven metabolic-associated fatty liver disease.

Table 2. Association between serum zinc levels and severe hepatic necro-inflammation analyzed by threshold effect.

FIGURE LEGENDS

Figure 1. Flowchart for the study.

Figure 2. Association between serum zinc levels and probability of having severe hepatic necro-inflammation (adjusted for age, sex, body mass index, levels of serum alanine aminotransferase, aspartate aminotransferase, gamma-glutamyltranspeptidase, glucose, triglycerides, total cholesterol and HOMA of insulin resistance) in patients with MAFLD. The y-axis represents the probability of severe hepatic necro-inflammation, and the slope of the curve reflects β (with 95%CIs), which represents a positive or a negative association.