Clin. Lab. 2019;65:XXX-XXX

ORIGINAL ARTICLE

Association between Serum Ferritin Level and Nonalcoholic Fatty Liver Disease in a Non-obese Chinese Population: a Cross-Sectional Study

Jinmei Yao [1, *], Yuying Dai [2, *], Juanwen Zhang [1], Xuyao Zhang [3], Ruoheng Zheng [2]

[*] *Jinmei Yao and Yuying Dai contributed equally to this work*
[1] *Department of Laboratory Medicine, The First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, China*
[2] *School of Clinical Medicine, Hangzhou Medical College, Hangzhou, China*
[3] *Clinical Medicine, Hangzhou Normal University Qianjiang College, Hangzhou, China*

SUMMARY

Background: **The aim of the study is to evaluate the cross-sectional association between serum ferritin level and nonalcoholic fatty liver disease (NAFLD) in a non-obese Chinese population.**
Methods: **A cross-sectional study was performed among 1,020 non-obese subjects (body mass index < 25 kg/m^2) who took their annual health examination at the First Affiliated Hospital, College of Medicine, Zhejiang University. Serum ferritin level and other clinical and laboratory parameters were measured in the population. Liver ultrasound examinations were performed to diagnose NAFLD.**
Results: **Of the 1,020 enrolled participants, 148 (14.51%) fulfilled the diagnostic criteria for NAFLD. Subjects with NAFLD had a higher level of serum ferritin than individuals without NAFLD in non-obese subjects. Serum ferritin level was significantly and positively correlated with parameters of MS (BMI, SBP, TG and FPG) in NAFLD group. Stepwise logistic regression analysis showed that serum ferritin level was significantly associated with the risk factor for NAFLD. After adjusting for confounders, serum ferritin level was an independent factor predicting advanced fibrosis (FIB-4 ≥ 1.3) in NAFLD participants.**
Conclusions: **Increased serum ferritin level is significantly associated with NAFLD, and elevated serum ferritin level is an independent factor predicting advanced fibrosis for NAFLD in a non-obese Chinese population.**
(Clin. Lab. 2019;65:xx-xx. DOI: 10.7754/Clin.Lab.2019.181250)

Correspondence:
Ruoheng Zheng
School of Clinical Medicine
Hangzhou Medical College
Binwen Road 481
310023 Hangzhou
Zhejiang Province
China
Phone/Fax: +86 571 87692696
Email: ruoruo1982@hotmail.com

Manuscript accepted February 2, 2019

KEY WORDS

nonalcoholic fatty liver disease, ferritin, insulin resistance, metabolic syndrome

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is defined as lipid deposition in hepatocytes without excessive alcohol drinking and other specific causes, with the progression spectrum ranging from nonalcoholic fatty liver (NAFL) to nonalcoholic steatohepatitis (NASH) and cirrhosis [1]. During the past two decades, NAFLD has grown to become the most prominent chronic liver diseases [2]. A striking increase in the prevalence of NAFLD in China has put it under the spotlight as a pub-

lic health problem. Approximately 20 - 30% of the general population are affected [3].

Although obesity is a well-known risk factor for predicting the development of NAFLD [4], accumulating clinical and epidemiological evidence indicated that NAFLD is also prevalent in non-obese individuals [5]. Of note, it has been noticed that lean-NAFLD [The Asian body mass index (BMI) cutoff of 25 kg/m^2 was used to define lean-NAFLD] seems to be more common in Asian-Pacific region than in Western countries [6]. For example in China, a recent community study by Wei et al. reported that the prevalence of NAFLD was as high as 19.3% in non-obese population [7]. The underlying mechanism of NAFLD reflects a complex interaction involving genetics, inflammatory cascade, and metabolic and oxidative stress-related factors. In addition to obesity, other potential factors that involve the development of NAFLD should be taken into account. Presently, the precise risk factors for the development of NAFLD in non-obese population remains unclear. Ferritin is a hollow globular protein with a molecular weight of about 460 kDa, consisting of highly conserved three-dimensional structures. Ferritin is crucial for host iron homeostasis which could act both as an iron carrier and iron storage protein [8]. The elevated serum ferritin is increasingly found to be closely related to insulin resistance and significantly associated with metabolic syndrome [9,10]. Zhang et al. found that participants with higher serum ferritin levels are more likely to have hyperuricemia [11]. The constellation of metabolic abnormalities are well-established risk factors involved in NAFLD. Moreover, the liver plays a key role in recycling iron as the organ synthesizes and stores ferritin, acting as the fundamental source of reserve iron in the body. Several cross-sectional and prospective studies also indicate a potential association between ferritin and NAFLD [12]. A recent large population-based study by Jung et al. from Korea observed that elevation of serum ferritin level was significantly associated with liver steatosis and fibrosis in NAFLD subjects [13]. We have previously revealed that serum ferritin values were higher and correlated positively with liver injury in HBV-related cirrhotic patients [14]. As far as we know, there is no clinical study to address the association between hyperferritinemia and lean-NAFLD. In this study, we aimed to explore the relationship between serum ferritin level and ultrasonography-proven NAFLD in non-obese Chinese subjects via a cross-sectional study.

MATERIALS AND METHODS

Ethics

The study was approved by the ethics committee of the First Affiliated Hospital, School of Medicine, Zhejiang University in China. Verbal informed consent was obtained from all subjects and recorded by the physician who explained the study procedures.

Subjects

Initially, our study included 1,208 participants who took their health checkup at the First Affiliated Hospital, College of Medicine, Zhejiang University between May 2017 and March 2018. Subjects who met the following criteria were excluded: BMI ≥ 25 kg/m^2; those with alcohol consumption > 140 g/week for men and > 70 g/week for women; those with a history of viral hepatitis, autoimmune hepatitis or other forms of chronic liver disease. The remaining 1,020 eligible subjects were enrolled in the current analysis. The diagnosis of hepatic steatosis was made according to the guideline presented by the Fatty Liver Disease Study Group of the Chinese Liver Disease Association based on abdominal ultrasound [15]. Specifically, hepatic steatosis was diagnosed according to characteristic echo patterns, such as diffuse hyperechogenicity of the liver relative to the kidneys, ultrasound beam attenuation, and poor visualization of intrahepatic structures.

Clinical examination and biochemical analyses

The baseline evaluations were conducted in the morning after an overnight fast using a standard manner as previously reported [16]. In brief, weight, height, and blood pressure were measured, and BMI was calculated as weight in kilograms divided by height in meters squared. Ultrasonic examination was carried out by an experienced ultrasonographist who was unaware of the patient details, using a Toshiba Nemio 20 sonography machine with a 3.5-MHz probe (Toshiba, Tokyo, Japan). Fasting blood samples were obtained from an antecubital vein and analyzed by clinical laboratory personnel. The parameters, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c), fasting plasma glucose (FPG), uric acid (UA), sialic acid (SA) and Fe were measured using a Hitachi 7600 clinical analyzer (Hitachi, Tokyo, Japan), hemoglobin (HGB) was determined using the Sysmex XE-2100 automated hematology analyzer (Sysmex Corp, Kobe, Japan). Serum ferritin levels were assessed using the chemiluminescence microparticle immunoassay Architect System i2000SR. The coefficients of variation were less than 8% for inter-assay and intra-assay. Elevation of ALT was defined as ALT level ≥ 40 IU/L for males and ALT level ≥ 35 IU/L for females. FIB-4 index was calculated according to the following equation: Age (years) x AST (U/L)/PLT (10^9/L) x $\sqrt{ALT\,(U/L)}$.

Statistical analyses

Statistical analyses were performed using SPSS, version 22 (IBM SPSS Statistics for Windows, IBM Corp., Armonk, NY, USA). The Kolmogorov-Smirnov test was used to assess whether continuous data were normally distributed. Data are presented as the mean ± standard deviation when data were found to be normally distributed or as the median and range if the distribution was skewed. Differences between the independent groups

Table 1. Characteristics of study participants with or without NAFLD in non-obese subjects.

Variables	Without NAFLD	With NAFLD	p-value
n (women)	872 (319)	148 (53)	0.357
n (premenopausal/postmenopausal)	218/101	35/18	0.739
Age (years)	43.4 ± 7.4	47.4 ± 5.9	< 0.001
BMI (kg/m^2)	21.0 ± 1.4	23.4 ± 1.1	< 0.001
SBP (mmHg)	121 ± 12	133 ± 11	< 0.001
DBP (mmHg)	75 ± 11	78 ± 10	0.071
ALT (U/L)	18 (6 - 47)	26 (7 - 197)	< 0.001
AST (U/L)	19 (11 - 40)	27 (12 - 130)	< 0.001
TG (mmol/L)	0.99 (0.39 - 1.60)	1.47 (0.54 - 18.05)	< 0.001
TC (mmol/L)	4.01 ± 0.88	4.67 ± 1.08	0.078
HDL-C (mmol/L)	1.50 ± 0.28	1.14 ± 0.22	< 0.001
FPG (mmol/L)	4.62 (3.81 - 6.01)	5.41 (3.82 - 16.46)	< 0.001
UA (umol/L)	290.7 ± 50.9	373 ± 41.0	< 0.001
SA (mg/dL)	50.1 ± 5.0	66.8 ± 6.5	< 0.001
HGB (g/L)	139.0 ± 12.0	153.6 ± 10.3	< 0.001
hsCRP (mg/L)	0.7 (0.2 - 5.7)	2.1 (1.3 - 19.2)	< 0.001
Fe (umol/L)	17.8 (7.4 - 30.1)	19.3 (8.1 - 96.1)	0.057
Ferritin (ng/mL)	89.3 (28.0 - 259.9)	276.7 (34.5 - 786.6)	< 0.001

were determined using the Student's *t*-test or the Mann-Whitney *U* test. Spearman's correlation analysis was used to examine the correlation between serum ferritin levels and parameters of metabolic syndrome. Stepwise logistic regression analysis was used to evaluate the risk factors for NAFLD. Multivariate logistic regression was used to evaluate the associations between serum ferritin and the advanced fibrosis in NAFLD subjects.

RESULTS

Clinical and laboratory characteristics of subjects
Of the 1,020 enrolled participants, 148 (14.51%) fulfilled the diagnostic criteria for NAFLD. The general demographic and biochemical characteristics of the subjects were shown in Table 1. There was no difference in terms of gender and menopausal status between the two groups. We found that participants with NAFLD had a higher BMI even within normal range than individuals without NAFLD in non-obese subjects. Furthermore, the subjects with NAFLD were relatively older and had higher levels of SBP, ALT, AST, TG, FPG, UA, SA, HGB, and hsCRP, but a lower level of HDL-C, compared with those without NAFLD (all $p < 0.05$). DBP, serum TC, and Fe levels appeared to trend higher in the NAFLD participants, but did not reach statistical significance. As illustrated in Table 1, serum ferritin levels were significantly higher in participants with NAFLD than those without NAFLD, suggesting a potential link between serum ferritin levels with NAFLD in non-obese subjects.

Comparison of serum ferritin in NAFLD participants with different serum ALT levels
We stratified NAFLD participants into two groups by their serum ALT levels: high (ALT level ≥ 40 IU/L for males and ALT level ≥ 35 IU/L for females) and low (ALT level < 40 IU/L for males and ALT level < 35 IU/L for females). Compared with the low serum ALT group, we found that the serum ferritin level was markedly higher in NAFLD participants in the high serum ALT group [310.9 (51.5 - 786.6) ng/mL vs. 220.7 (34.5-521.6), $p < 0.001$)].

Risk factors analysis for NAFLD in non-obese subjects
A stepwise logistic regression analysis was performed to explore the relationship between serum ferritin and NAFLD. Seventeen variables including age, gender, BMI, SBP, DBP, ALT, AST, TG, TC, HDL-C, FPG, UA, SA, HGB, hsCRP, Fe, and ferritin were entered into the original equation. Further analyses revealed that seven variables, age, BMI, ALT, FPG, UA, HGB, hsCRP, and ferritin, were significantly and positively associated with the risk for NAFLD (Table 2).

Table 2. Risk factors associated with the presence of NAFLD in non-obese subjects

Variables	β	OR	95% CI	p-value
Age	0.117	1.099	1.048 - 1.146	< 0.001
BMI	0.350	1.451	1.247 - 1.643	< 0.001
ALT	0.087	1.056	1.012 - 1.109	0.015
FPG	0.105	1.071	1.034 - 1.108	< 0.001
UA	0.190	1.118	1.059 - 1.172	< 0.001
HGB	0.042	1.007	1.001 - 1.013	0.028
hsCRP	0.181	1.108	1.050 - 1.164	< 0.001
Ferritin	0.203	1.122	1.066 – 1.182	< 0.001

Table 3. Correlations between serum ferritin and parameters of metabolic syndrome in non-obese subjects with NAFLD.

	BMI	SBP	DBP	TG	HDL-C	FPG
r-value	0.259	0.223	0.120	0.282	-0.104	0.303
p-value	< 0.001	< 0.001	0.089	< 0.001	0.071	< 0.001

Table 4. Independent predictors of advanced fibrosis of NAFLD in non-obese subjects according to serum ferritin in unadjusted and adjusted models.

Model	Odds Ratio (95% CI)	p-value
Unadjusted	2.760 (2.169 - 3.342)	< 0.001
Adjusted for age, gender, and BMI	1.898 (1.163 - 2.621)	< 0.001
Adjusted for age, gender, BMI, UA, and hsCRP	1.720 (1.149 - 2.302)	< 0.001
Adjustment for age, gender, BMI, UA, hsCRP, and HGB	1.401 (1.091 - 1.714)	0.002

Serum ferritin levels are correlated with parameters of metabolic syndrome in non-obese subjects with NAFLD

We performed a correlation analysis to determine the associations between serum ferritin levels and parameters of metabolic syndrome in non-obese subjects with NAFLD. Our results showed that the serum ferritin levels were significantly and positively correlated with BMI ($r = 0.259$, $p < 0.001$), SBP ($r = 0.223$, $p < 0.001$), TG ($r = 0.282$, $p < 0.001$), FPG ($r = 0.303$, $p < 0.001$). The constellation of metabolic syndrome is strongly associated with an increased risk of NAFLD in a general population. Thus, NAFLD is considered to be the hepatic manifestation of metabolic syndrome. These results indirectly support that serum ferritin may be a significant risk factor for NAFLD in non-obese subjects.

Relationship between serum ferritin level and advanced fibrosis of NAFLD in non-obese subjects

NAFLD participants were further divided into two groups according to their FIB-4 score: low risk of fibrosis (FIB-4 < 1.3) and advanced fibrosis (FIB-4 ≥ 1.3). Serum ferritin levels in the advanced fibrosis group were significantly higher than those in the low risk fibrosis group [308.8 (64.1 - 786.6) ng/mL vs. 239.0 (34.5 - 326.6) ng/mL, $p < 0.001$]. The results of unadjusted and adjusted multivariate logistic regression analysis models were shown in Table 4. Following adjustment for age, gender, BMI, UA, hsCRP, and HGB (all $p < 0.05$) selected by stepwise regression, our results showed that serum ferritin levels were an independent factor predicting advanced fibrosis (FIB-4 ≥ 1.3) in NAFLD participants, the OR was 1.401 (95% CI: 1.091 - 1.714, $p = 0.002$).

DISCUSSION

Several studies showed that the serum ferritin level was positively and independently associated with the presence and severity of NAFLD [17,18]. On the contrary, another large population-based study proposed that serum ferritin level has low diagnostic accuracy for specifically detecting liver fibrosis in patients with NAFLD [19]. These controversial data may arise from sample size, differences in study population and definition of illness course. However, the association of serum ferritin level with NAFLD has not yet been fully clarified among the non-obese population. In this cross-sectional study, we found that the prevalence of NAFLD was 14.51% in a non-obese Chinese population. Our results further clearly showed that NAFLD participants had higher serum ferritin levels, and hyperferritinemia is positively associated with the increased risk for NAFLD. We believe that this retrospective study, for the first time, demonstrates a significant correlation between serum ferritin levels and NAFLD in non-obese individuals.

Though the detailed mechanism by which ferritin is involved in NAFLD remains to be elucidated, several possible explanations have been proposed. First, insulin resistance served as the first hit plays a fundamental role in NAFLD pathogenesis [20]. Growing evidence has revealed a statistically-significant interplay between serum ferritin and insulin resistance. In Korean postmenopausal women, prevalence of insulin resistance and metabolic syndrome showed a significant increasing trend according to the serum ferritin quartiles [21]. Zelber-Sagi et al. demonstrated that NAFLD and hyperinsulinemia are major determinants of serum ferritin levels [22]. The second key mechanism associated with the relationship between ferritin and NAFLD may be oxidative stress. Ferritin could stimulate the release of the toxic free radicals to promote oxidative stress by the Fenton reaction and then be involved in the pathogenesis of NAFLD [23]. The third, as we know, ferritin is a well-documented acute-phase protein [24]. Ferritin is speculated to be a proinflammatory signaling molecule by activating nuclear factor kappaB pathways in hepatic stellate cells and macrophages and, in turn, its secretion was increased in response to a variety of proinflammatory cytokines and chemokines [25].

Simple steatosis is traditionally considered relatively benign, but nearly 10 to 25% of cases may progress to more aggressive NASH. Up to one-third of the patients with NASH are prone to the risk of severe liver fibrosis, cirrhosis, and end-stage liver disease [26]. Currently, liver biopsy is considered as the gold standard for evaluation and staging of hepatic fibrosis, but it has well-known drawbacks, including invasiveness, sampling errors, inter-/intra-observer variability and cost [27]. Non-invasive modalities are widely used to identify advanced liver fibrosis of NAFLD among individuals [28]. Our previous meta-analysis study reported that FIB-4 score, a simple non-invasive index composed of readily available routine laboratory tests is valuable for staging liver fibrosis in chronic liver disease [29]. It has also been well demonstrated that FIB-4 score was suitable for predicting advanced fibrosis in patients with NAFLD [30]. In this study, we first reported that serum ferritin levels were an independent factor predicting advanced fibrosis (FIB-4 $\geq$ 1.3) of NAFLD in non-obese subjects following adjusting for confounders (OR 1.606 (95% CI: 1.117 - 2.108). Another Swedish cohort with a long-term follow-up of 16 years also revealed that biopsy-proven NAFLD patients with high serum ferritin levels had more advanced fibrosis while hyperferritinemia was strongly associated with subsequent higher mortality [31].

Several limitations exist in this study that should be acknowledged. First, liver ultrasonographic examinations were performed to diagnose NAFLD instead of the liver biopsy, which is not sensitive enough to detect subjects with mild steatosis. Nevertheless, it is more practical with acceptable power of diagnosis in epidemiological studies of NAFLD. Second, several potential confounders, including iron intake, protein, and inflammatory markers have not been analyzed in this study. Third, whether elevated serum ferritin is a bystander, a cause or a consequence of NAFLD cannot be concluded from the results of this cross-sectional study. Further large prospective studies are needed to verify the relationship between ferritin and NAFLD.

CONCLUSION

In summary, this cross-sectional study demonstrated the significant association between serum ferritin and NAFLD in non-obese subjects. This finding may help to get a better understanding of the involved mechanisms and open up new avenues for the prevention of NAFLD.

Author Contributions:

Jinmei Yao and Juanwen Zhang performed the majority of data collection and partial analysis; Xuyao Zhang carried out major data analysis and statistical analysis; Yuying Dai and Ruoheng Zheng designed the study and wrote the manuscript.

Declaration of Interest:

This study is supported by the Natural Science Foundation of Zhejiang province (LY17H030007 and LY19H0 200028) and the Health Bureau Research Foundation of Zhejiang province (2016KYB085 and 2016KYB102). The funding sources had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References:

1. Leoni S, Tovoli F, Napoli L, Serio I, Ferri S, Bolondi L. Current guidelines for the management of non-alcoholic fatty liver disease: A systematic review with comparative analysis. World J Gastroenterol 2018;24:3361-73 (PMID: 30122876).

2. Aller R, Fernández-Rodríguez C, Lo Iacono O, et al. Consensus document. Management of non-alcoholic fatty liver disease (NAFLD). Clinical practice guideline. Gastroenterol Hepatol 2018;41:328-49 (PMID: 29631866).

3. Wang H, Gu Y, Zheng L, et al. Association between bedtime and the prevalence of newly diagnosed non-alcoholic fatty liver disease in adults. Liver Int 2018;38:2277-86 (PMID: 29851261).

4. Soderborg TK, Clark SE, Mulligan CE, et al. The gut microbiota in infants of obese mothers increases inflammation and susceptibility to NAFLD. Nat Commun 2018;9:4462 (PMID: 30367045).

5. Lu FB, Hu ED, Xu LM, et al. The relationship between obesity and the severity of non-alcoholic fatty liver disease: systematic review and meta-analysis. Expert Rev Gastroenterol Hepatol 2018;12:491-502 (PMID: 29609501).

6. Cho HC. Prevalence and Factors Associated with Nonalcoholic Fatty Liver Disease in a Nonobese Korean Population. Gut Liver 2016;10:117-25 (PMID: 26260755).

7. Wei JL, Leung JC, Loong TC, et al. Prevalence and Severity of Nonalcoholic Fatty Liver Disease in Non-Obese Patients: A Population Study Using Proton-Magnetic Resonance Spectroscopy. Am J Gastroenterol 2015;110:1306-14 (PMID: 26215532).

8. Foka P, Dimitriadis A, Karamichali E, et al. Alterations in the iron homeostasis network: A driving force for macrophage-mediated hepatitis C virus persistency. Virulence 2016;7(6):679-90 (PMID: 27058404).

9. Yoon H, Kim YS, Lee JH, Gi MY, Cha JA, Seong JM. Gender difference in the relationship between the ferritin and homeostasis model assessment of insulin resistance in non-diabetic Korean adults. PLoS One 2018;13(6):e0199465 (PMID: 29949646).

10. Suárez-Ortegón MF, Ensaldo-Carrasco E, Shi T, McLachlan S, Fernández-Real JM, Wild SH. Ferritin, metabolic syndrome and its components: A systematic review and meta-analysis. Atherosclerosis 2018;275:97-106 (PMID: 29886355).

11. Zhang YN, Xu C, Xu L, et al. High serum ferritin levels increase the risk of hyperuricemia: a cross-sectional and longitudinal study. Ann Nutr Metab 2014;64:6-12 (PMID: 24642593).

12. Du SX, Lu LL, Geng N, et al. Association of serum ferritin with non-alcoholic fatty liver disease: a meta-analysis. Lipids Health Dis 2017;16:228 (PMID: 29197393).

13. Jung JY, Shim JJ, Park SK, et al. Serum ferritin level is associated with liver steatosis and fibrosis in Korean general population. Hepatol Int. Epub 2018 Sep 4 (PMID: 30182166).

14. Mao W, Hu Y, Lou Y, Chen Y, Zhang J. Abnormal serum iron markers in chronic hepatitis B virus infection may be because of liver injury. Eur J Gastroenterol Hepatol 2015;27:130-6 (PMID: 25419642).

15. Zhang J, Zhao Y, Xu C, et al. Association between serum free fatty acid levels and nonalcoholic fatty liver disease: a cross-sectional study. Sci Rep 2014;4:5832 (PMID: 27058404).

16. Zhang J, Liu Z, Zhang X, Zhang L, Jin X. Association between urine retinol-binding protein levels and nonalcoholic fatty liver disease: A cross-sectional study in Chinese population. J Clin Lab Anal 2018;32(4):e22359 (PMID: 29194759).

17. Kim HB, Lee HS, Lee YJ. Association of serum ferritin levels with non-alcoholic fatty liver disease in postmenopausal women. Climacteric 2018;2:509-14 (PMID: 30106314).

18. Haghgoo SM, Sharafi H, Alavian SM. Serum cytokines, adipokines and ferritin for non-invasive assessment of liver fibrosis in chronic liver disease: a systematic review. Clin Chem Lab Med 2018 Sep 19 (PMID: 30231008).

19. Angulo P, George J, Day CP, et al. Serum ferritin levels lack diagnostic accuracy for liver fibrosis in patients with nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol 2014;12:1163-9 (PMID: 24342745).

20. Sookoian S, Pirola CJ. Systematic review with meta-analysis: risk factors for non-alcoholic fatty liver disease suggest a shared altered metabolic and cardiovascular profile between lean and obese patients. Aliment Pharmacol Ther 2017;46(2):85-95 (PMID: 28464369).

21. Cho MR, Park JK, Choi WJ, Cho AR1, Lee YJ. Serum ferritin level is positively associated with insulin resistance and metabolic syndrome in postmenopausal women: A nationwide population-based study. Maturitas 2017;103:3-7 (PMID: 28778329).

22. Zelber-Sagi S, Nitzan-Kaluski D, Halpern Z, Oren R. NAFLD and hyperinsulinemia are major determinants of serum ferritin levels. J Hepatol 2007;46:700-7 (PMID: 17150278).

23. Arosio P, Ingrassia R, Cavadini P. Ferritins: a family of molecules for iron storage, antioxidation and more. Biochim Biophys Acta 2009;1790:589-99 (PMID: 18929623).

24. Uysal S, Armutcu F, Aydogan T, Akin K, Ikizek M, Yigitoglu MR. Some inflammatory cytokine levels, iron metabolism and oxidan stress markers in subjects with nonalcoholic steatohepatitis. Clin Biochem 2011;44:1375-9 (PMID: 22015687).

25. Pisano G, Lombardi R, Fracanzani AL. Vascular Damage in Patients with Nonalcoholic Fatty Liver Disease: Possible Role of Iron and Ferritin. Int J Mol Sci 2016;17(5) (PMID: 27164079).

26. Li B, Zhang C, Zhan YT. Nonalcoholic Fatty Liver Disease Cirrhosis: A Review of Its Epidemiology, Risk Factors, Clinical Presentation, Diagnosis, Management, and Prognosis. Can J Gastroenterol Hepatol 2018;2018:2784537 (PMID: 30065915).

27. Itoh Y, Seko Y, Shima T, et al. Accuracy of non-invasive scoring systems for diagnosing non-alcoholic steatohepatitis-related fibrosis: Multicenter validation study. Hepatol Res 2018;48:1099-107 (PMID: 29974624).

28. Vilar-Gomez E, Chalasani N. Non-invasive assessment of non-alcoholic fatty liver disease: Clinical prediction rules and blood-based biomarkers. J Hepatol 2018;68:305-15 (PMID: 29154965).

29. Li Y, Chen Y, Zhao Y. The diagnostic value of the FIB-4 index for staging hepatitis B-related fibrosis: a meta-analysis. PLoS One 2014;9:e105728 (PMID: 25165830).

30. Sumida Y, Yoneda M, Hyogo H, et al. Validation of the FIB4 index in a Japanese nonalcoholic fatty liver disease population. BMC Gastroenterol 2012;12:2 (PMID: 22221544).

31. Hagström H, Nasr P, Bottai M, et al. Elevated serum ferritin is associated with increased mortality in non-alcoholic fatty liver disease after 16 years of follow-up. Liver Int 2016;36:1688-95 (PMID: 27064133).