



Trabajo Original

Associations between serum ferritin, iron, and liver transaminases in adolescents: a large cross-sectional study

Asociaciones entre la ferritina sérica, el hierro y las transaminasas hepáticas en adolescentes: un amplio estudio transversal

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Abstract

Introduction: few previous studies suggest that serum iron status may be associated with liver function, but the relevant evidence remains limited, especially in adolescents.

Objective: we aimed to investigate the association between serum ferritin, iron, and liver transaminases in adolescents.

Methods: a cross-sectional study including 3,404 adolescents aged 10-19 was performed based on the National Health and Nutrition Examination Survey. Weighted multivariate regression, subgroup analysis, and sensitivity analysis were used.

Results: a total of 3,404 adolescents were eventually included. Serum ferritin and iron were positively correlated to alanine aminotransferase (ALT) and aspartate aminotransferase (AST). The association between serum ferritin and ALT remained positive in all genders and races, but only remained positive in girls and several races between serum ferritin and AST. The positive correlations kept present among girls between serum iron and ALT, and also kept present among girls and non-Hispanic whites between serum iron and AST. Additionally, serum ferritin and iron were also positively correlated to elevated ALT and elevated AST using binary logistic regression analysis. After excluding the subjects with serum ferritin levels above the upper limit of normal, the main results remained the same basically.

Conclusion: the present results add novel evidences about the associations between serum ferritin, iron, and liver transaminases, which requires more confirmatory studies.

Keywords:

Ferritins. Iron. Liver transaminases. Adolescents. Nutrition surveys.

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Data availability statement: the data used and analyzed in the present study are publicly available from <http://www.cdc.gov/nchs/nhanes/>.

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Resumen

Introducción: pocos estudios previos sugieren que el estado del hierro sérico pueda estar asociado con la función hepática, pero la evidencia relevante sigue siendo limitada, especialmente en adolescentes.

Objetivo: nuestro objetivo fue investigar la asociación entre la ferritina sérica, el hierro y las transaminasas hepáticas en adolescentes.

Métodos: se realizó un estudio transversal que incluyó a 3,404 adolescentes de diez a 19 años de edad, basado en la Encuesta Nacional de Examen de Salud y Nutrición. Se utilizaron la regresión multivariada ponderada, el análisis de subgrupos y el análisis de sensibilidad.

Resultados: finalmente, se incluyó un total de 3.404 adolescentes. La ferritina sérica y el hierro se correlacionaron positivamente con la alanina aminotransferasa (ALT) y la aspartato aminotransferasa (AST). La asociación entre ferritina sérica y ALT se mantuvo positiva en todos los géneros y razas, pero solo se mantuvo positiva en niñas y en varias razas entre ferritina sérica y AST. Las correlaciones positivas siguieron presentes en las niñas entre el hierro sérico y la ALT, y también en las niñas y personas blancas no hispanas entre el hierro sérico y la AST. Además, la ferritina sérica y el hierro también se correlacionaron positivamente con ALT elevada y AST elevada mediante análisis de regresión logística binaria. Después de excluir a los sujetos con niveles de ferritina sérica por encima del límite superior de la normalidad, los resultados principales se mantuvieron básicamente iguales.

Conclusión: los presentes resultados agregan evidencias novedosas sobre las asociaciones entre la ferritina sérica, el hierro y las transaminasas hepáticas, lo que requiere más estudios confirmatorios.

Palabras clave:

Ferritinas. Hierro.
Transaminasas hepáticas.
Adolescentes. Encuestas
de nutrición.

INTRODUCTION

Liver function damage can be caused by various factors, such as viruses, drugs, heavy metals, and immune factors. Recently, the prevalence of liver function damage in adolescents has increased, resulting in an increasing burden for the health care system (1). Unexplained childhood hepatitis has been rampant in many countries, which can lead to elevated transaminases, including alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Additionally, the incidence of nonalcoholic liver disease in American adolescents increased 2.7-fold from 1988 to 2010 (2). However, liver impairment in adolescents is often overlooked, possibly due to a lack of routine physical examination and insufficient attention to adolescent health, leading to delays in diagnosis and intervention. Therefore, it is crucial to better understand the potential risk factors for liver function damage, especially in adolescents.

Iron is an essential metal element in our body, and ferritin, an iron storage protein, is essential for cellular iron homeostasis. Ferritin can sequester excess intracellular iron and store it in a redox-inactive form. In addition to iron status, systemic ferritin levels are important indicators of immunological and inflammatory disorders (3,4). Aberrant iron homeostasis or iron distribution may be involved in liver dysfunction, so serum iron may be a potential marker for liver function (5,6). Several studies have indicated that high levels of serum ferritin may be associated with liver diseases in adults (7,8). Additionally, a previous study showed an independent association between elevated serum ferritin and elevated ALT in Korean children and adolescents (9), and another study indicated that serum ferritin was correlated with liver fat in male adolescents with obesity (10). However, a meta-analysis showed that the connection between serum ferritin and non-alcoholic steatohepatitis against non-alcoholic fatty liver group in pediatric or adolescent populations was inconsistent with adults (11). Therefore, the correlation between serum ferritin levels and liver function in adolescents remains to be further explored since different populations, such as those from different countries or with different states, may affect the results. The present study aims to investigate the association of serum levels of ferritin and

iron with liver transaminases (serum ALT and AST) using a representative sample of adolescents from the National Health and Nutrition Examination Survey (NHANES).

METHODS

STUDY POPULATION

The present study included adolescents aged ten to 19 years old from NHANES 2005-2010 and 2015-2018. NHANES is a cross-sectional survey that provides a large amount of information about nutrition and health of the United States population, and its details have been previously described (12). The present study is a cross-sectional analysis which was approved by the Ethical Review Committee of the National Center for Health Statistics. After excluding those whose age did not meet the requirements, those who were pregnant, those without the necessary data, and those with high blood pressure, diabetes, hepatitis B surface antigen (+) or hepatitis C RNA (+), a total of 3,404 adolescents were eventually included in the present study (Fig. 1).

EXPOSURE AND OUTCOME VARIABLES

The exposure variables are serum ferritin and iron, and the outcome variables are ALT and AST. According to the NHANES, serum ferritin was measured using the Roche/Hitachi 912 clinical analyzer in 2005-2008, the Roche Elecsys® 170 clinical analyzer in 2009-2010 and 2015, and the Roche Cobas® e601 in 2016-2018. Iron concentration was measured by a timed-end-point method using the Beckman Synchron LX20 or Beckman UniCel® Dx C800 Synchron in 2005-2010 and 2015-2016, and measured using the Roche Cobas® 6000 (c501 module) analyzer in 2017-2018. In this method, the system monitors absorbance changes at 560 nm at fixed-time intervals and these changes in absorbance are proportional to the iron concentration. The activity of serum ALT and AST was measured by an enzymatic rate method or a kinetic rate method using the Beckman Synchron LX20

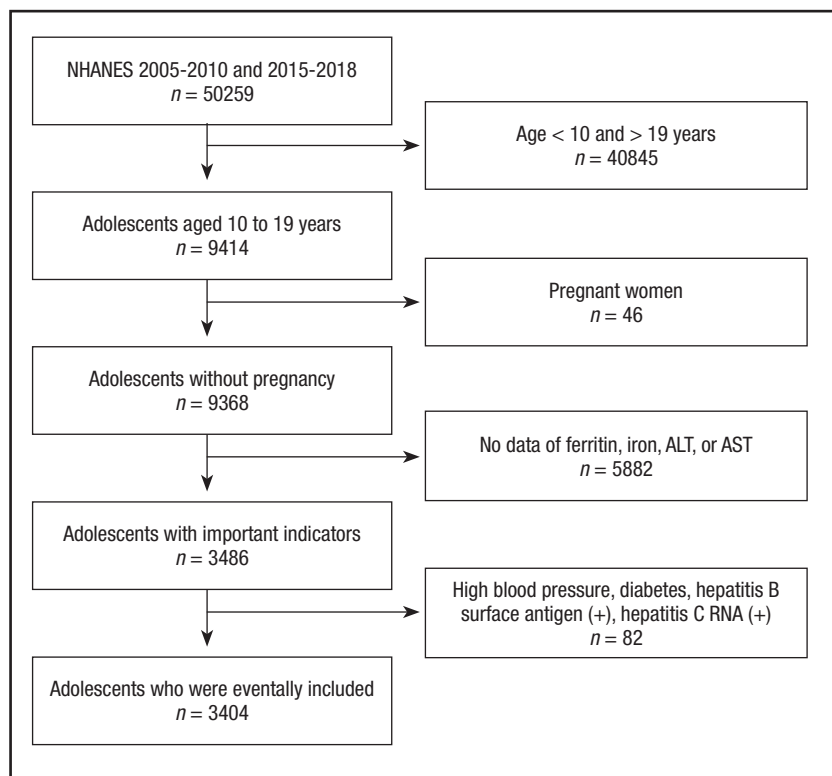


Figure 1. Flow diagram of participants selection. ALT: alanine aminotransferase; AST: aspartate aminotransferase.

or the Beckman UniCel® DxC800 Synchron. The system monitors absorbance changes at 340 nm at fixed-time intervals and these changes in absorbance are proportional to the activity. According to the guidelines from the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition (NASPGHAN), ALT \geq 26 U/l in male adolescents and \geq 22 U/l in female adolescents were defined as elevated ALT, and AST \geq 37 U/l was defined as elevated AST. More details are available from <http://www.cdc.gov/nchs/nhanes/>.

OTHER COVARIABLES

Several covariates were included as potential confounders, including age, body mass index (BMI), race (Mexican American, other Hispanic, non-Hispanic white, non-Hispanic black, and other races), education (less than high school, high school or equivalent, and more than high school), family income-poverty ratio (< 1, \geq 1 and < 3, \geq 3, and unknown), and smoking exposure. Therein, the smoking exposure was reflected by serum cotinine levels: a cotinine level below the detection limit was defined as “unexposed”, a cotinine level between the detection limit and 10 ng/ml was defined as “low-exposure”, and a cotinine level \geq 10 ng/ml was defined as “high-exposure” (13). Additionally, participants with high blood pressure, diabetes, hepatitis B surface antigen (+), and hepatitis C RNA (+) were excluded. Therein, high blood pressure and diabetes were defined as positive responses to the questions “Have you been told by a doctor or other health profes-

sional that you had hypertension, also called high blood pressure?” and “Have you ever been told by a doctor or health professional that you have diabetes?”, respectively. Hepatitis B surface antigen was measured by an immunometric immunoassay using the VITROS™ HBsAg Reagent Pack and VITROS™ Immunodiagnostic Products HBsAg Calibrator on the VITROS™ ECi/ECiQ Immunodiagnostic Systems and the VITROS™ 3600 Immunodiagnostic System, or an AUSZYME Monoclonal test. The quantitation of hepatitis C virus RNA was performed using the COBAS® AMPLICOR® HCV MONITOR Test on the COBAS® AMPLICOR® Analyzer. A detailed description of these methods can be found on the NHANES.

STATISTICAL ANALYSIS

NHANES sample weight was used to calculate all estimates. The continuous variables were expressed as the mean \pm standard deviation and the categorical variables were expressed as number (percentage). Weighted linear regression models or weighted Chi-squared tests were used to evaluate the differences of indicators between different groups. Weighted multivariate linear regression models were used to estimate the association of the levels of serum ferritin and iron with ALT and AST. Multivariate logistic regression models were used to investigate the association of serum ferritin and iron with elevated ALT and elevated AST. Packages R (<http://www.R-project.org>) and EmpowerStats software (<http://www.empowerstats.com>) were used for all of the data analyses. Statistical significance was set at $p < 0.05$.

RESULTS

CHARACTERISTICS OF THE PARTICIPANTS

A total of 3,404 adolescents, including 480 boys and 2,924 girls, were eligible for the present study. Table I shows the characteristics of the study population. The population included 26.23 % Mexican Americans, 8.93 % other Hispanics, 28.76 % non-Hispanic whites, 24.79 % non-Hispanic blacks, and 11.28 % other races. There were no statistical differences in basic characteristics between boys and girls, except for race.

RELATIONSHIPS BETWEEN SERUM FERRITIN LEVEL AND ALT AND AST

We built three models in the multivariate regression analyses: model 1 (unadjusted), model 2 (adjusted for gender, age, and race), and model 3 (adjusted for gender, age, race, education, family income-poverty ratio, BMI, and smoking exposure). According to the

results in table II, the serum ferritin levels were positively correlated to ALT in the model 1 ($\beta=0.08$, 95 % CI: 0.07-0.09, $p < 0.001$), the model 2 ($\beta=0.08$, 95 % CI: 0.07-0.09, $p < 0.001$), and the model 3 ($\beta=0.07$, 95 % CI: 0.06-0.08, $p < 0.001$). Then, we obtained the quartiles of ferritin levels (first quartile: 20.00 ng/ml, second quartile: 33.70 ng/ml, and third quartile: 53.00 ng/ml) and divided the ferritin levels into Q1, Q2, Q3, and Q4. We found that the p for trend was less than 0.001 in the three models, but only the Q4 of ferritins and ALT levels showed significant positive correlations ($p < 0.001$). In the subgroup analyses, the associations of serum ferritin with ALT remained positive in all genders and races.

Table III shows the association between serum ferritin and AST. The serum ferritin levels were also positively correlated to AST levels in the three models ($\beta=0.03$, 95 % CI: 0.02-0.04, $p < 0.001$). For ferritin quartiles, also only the Q4 was positively correlated with AST levels ($p < 0.001$). However, the associations of serum ferritin with AST remained positive only in girls and some races, including Mexican American, non-Hispanic white, and non-Hispanic black, in the subgroup analyses.

Table I. Characteristics of the study population

	All (n = 3,404)	Boys (n = 480)	Girls (n = 2,924)	p value
Age (years)	15.43 ± 2.25	15.15 ± 2.23	15.34 ± 2.28	0.088
BMI (kg/m ²)	24.51 ± 6.20	23.69 ± 5.56	23.98 ± 5.86	0.302
Ferritin (ng/ml)	41.48 ± 36.17	42.49 ± 33.43	42.20 ± 34.44	0.861
Iron (ug/dl)	79.06 ± 37.46	84.74 ± 40.55	82.66 ± 38.03	0.267
ALT (U/l)	17.22 ± 14.52	16.80 ± 13.34	16.71 ± 12.41	0.882
AST (U/l)	21.53 ± 9.68	21.64 ± 9.84	21.30 ± 8.00	0.393
<i>Race (%)</i>				
Mexican American	26.23	18.95	26.51	< 0.001
Other Hispanic	8.93	6.15	9.04	
Non-Hispanic white	28.76	33.57	28.97	
Non-Hispanic black	24.79	16.27	24.62	
Other races	11.28	25.06	10.86	
<i>Education (%)</i>				
Less than high school	83.81	84.70	84.15	0.702
High school or equivalent	10.08	9.64	9.22	
More than high school	6.11	5.66	6.63	
<i>Family income-poverty ratio (%)</i>				
< 1	27.50	16.51	20.72	0.197
≥ 1 and < 3	39.78	38.30	36.24	
≥ 3	24.71	37.80	36.02	
Unknown	8.02	7.39	7.01	
<i>Smoking exposure (%)</i>				
Unexposed	27.85	29.77	30.06	0.894
Low-exposure	64.92	62.03	62.35	
High-exposure	7.23	8.20	7.59	

Mean ± SD for continuous variables; p values were calculated by weighted linear regression model; (%) for categorical variables; p values were calculated by weighted Chi-squared test. All p values were used for comparisons between different genders. ALT: alanine aminotransferase; AST: aspartate aminotransferase; BMI: body mass index.

Table II. Association between serum ferritin and ALT

	Model 1, β (95 % CI)	Model 2, β (95 % CI)	Model 3, β (95 % CI)
Ferritin	0.08 (0.07, 0.09) [‡]	0.08 (0.07, 0.09) [‡]	0.07 (0.06, 0.08) [‡]
Quartiles of ferritin			
Q1 (lowest quartile)	Reference	Reference	Reference
Q2	-0.10 (-1.33, 1.12)	-0.11 (-1.34, 1.12)	0.46 (-0.75, 1.67)
Q3	0.79 (-0.41, 1.98)	0.82 (-0.38, 2.02)	1.06 (-0.12, 2.23)
Q4	4.63 (3.42, 5.83) [‡]	4.62 (3.41, 5.83) [‡]	4.50 (3.32, 5.69) [‡]
<i>p</i> for trend	< 0.001	< 0.001	< 0.001
Stratified by gender[§]			
Boys	0.04 (0.01, 0.08) [*]	0.04 (0.00, 0.08) [*]	0.04 (0.00, 0.07) [*]
Girls	0.08 (0.07, 0.10) [‡]	0.08 (0.07, 0.10) [‡]	0.08 (0.06, 0.09) [‡]
Stratified by race[§]			
Mexican American	0.07 (0.05, 0.09) [‡]	0.07 (0.05, 0.09) [‡]	0.07 (0.05, 0.09) [‡]
Other Hispanic	0.05 (0.02, 0.08) [‡]	0.05 (0.02, 0.08) [‡]	0.04 (0.01, 0.07) [‡]
Non-Hispanic white	0.08 (0.07, 0.10) [‡]	0.08 (0.06, 0.10) [‡]	0.08 (0.06, 0.09) [‡]
Non-Hispanic black	0.07 (0.05, 0.10) [‡]	0.07 (0.05, 0.10) [‡]	0.07 (0.04, 0.09) [‡]
Other races	0.10 (0.04, 0.16) [‡]	0.10 (0.04, 0.16) [‡]	0.10 (0.04, 0.17) [‡]

**p* < 0.05, [†]*p* < 0.01, [‡]*p* < 0.001. Model 1 was not adjusted. Model 2 was adjusted for gender, age, and race. Model 3 was adjusted for gender, age, race, education, family income-poverty ratio, BMI, and smoking exposure. [§]In the subgroup analyses stratified by gender or race, the model was not adjusted for the stratification variables themselves. ALT: alanine aminotransferase; BMI: body mass index; CI: confidence interval.

Table III. Association between serum ferritin and AST

	Model 1, β (95 % CI)	Model 2, β (95 % CI)	Model 3, β (95 % CI)
Ferritin	0.03 (0.02, 0.04) [‡]	0.03 (0.02, 0.04) [‡]	0.03 (0.02, 0.04) [‡]
Quartiles of ferritin			
Q1 (lowest quartile)	Reference	Reference	Reference
Q2	0.07 (-0.74, 0.89)	0.03 (-0.79, 0.85)	0.08 (-0.74, 0.90)
Q3	0.78 (-0.02, 1.58)	0.75 (-0.05, 1.55)	0.79 (-0.01, 1.59)
Q4	2.01 (1.20, 2.81) [‡]	2.02 (1.21, 2.82) [‡]	2.07 (1.27, 2.88) [‡]
<i>p</i> for trend	< 0.001	< 0.001	< 0.001
Stratified by gender[§]			
Boys	0.01 (-0.01, 0.04)	0.01 (-0.01, 0.04)	0.02 (-0.01, 0.04)
Girls	0.03 (0.02, 0.04) [‡]	0.03 (0.02, 0.04) [‡]	0.03 (0.02, 0.04) [‡]
Stratified by race[§]			
Mexican American	0.03 (0.02, 0.04) [‡]	0.03 (0.02, 0.04) [‡]	0.03 (0.02, 0.04) [‡]
Other Hispanic	0.02 (-0.01, 0.05)	0.02 (-0.01, 0.05)	0.01 (-0.02, 0.05)
Non-Hispanic white	0.04 (0.03, 0.06) [‡]	0.04 (0.03, 0.06) [‡]	0.04 (0.03, 0.06) [‡]
Non-Hispanic black	0.02 (0.01, 0.04) [‡]	0.02 (0.01, 0.04) [‡]	0.02 (0.01, 0.04) [‡]
Other races	0.02 (-0.01, 0.06)	0.02 (-0.01, 0.06)	0.03 (-0.00, 0.07)

**p* < 0.05, [†]*p* < 0.01, [‡]*p* < 0.001. Model 1 was not adjusted. Model 2 was adjusted for gender, age, and race. Model 3 was adjusted for gender, age, race, education, family income-poverty ratio, BMI, and smoking exposure. [§]In the subgroup analyses stratified by gender or race, the model was not adjusted for the stratification variables themselves. AST: aspartate aminotransferase; BMI: body mass index; CI: confidence interval.

RELATIONSHIPS BETWEEN SERUM IRON LEVEL AND ALT AND AST

As shown in table IV, the serum iron levels were positively correlated to ALT in the model 1 ($\beta = 0.01$, 95 % CI: 0.00-0.02, $p < 0.05$), the model 2 ($\beta = 0.01$, 95 % CI: 0.00-0.03, $p < 0.05$), and the model 3 ($\beta = 0.03$, 95 % CI: 0.02-0.04, $p < 0.001$). The Q4 of iron levels and ALT levels also showed significant positive correlations in the three models and all the p for trend were less than 0.05. In the subgroup analyses, the associations of serum iron with ALT remained positive in the girls, but there were few significant associations across race stratification.

Table V shows the association between serum iron and AST. The serum iron levels were also positively correlated to AST lev-

els in the three models ($\beta = 0.01$, 95 % CI: 0.00-0.02, $p < 0.01$). For iron quartiles, the Q3 and Q4 were positively correlated with AST levels in the three models, and the p for trend was 0.005, 0.005, and < 0.001 , respectively. The associations of serum iron with AST remained positive only in the girls and the non-Hispanic white in the subgroup analyses.

RELATIONSHIPS BETWEEN SERUM FERRITIN, IRON, ELEVATED ALT AND ELEVATED AST

Whether it is serum ferritin or iron, their levels were positively correlated with elevated ALT and elevated AST in the three models according to the results of binary logistic regression analysis (Table VI).

Table IV. Association between serum iron and ALT

	Model 1, β (95 % CI)	Model 2, β (95 % CI)	Model 3, β (95 % CI)
Iron	0.01 (0.00, 0.02)*	0.01 (0.00, 0.03)*	0.03 (0.02, 0.04)†
Quartiles of iron			
Q1 (lowest quartile)	Reference	Reference	Reference
Q2	0.65 (-0.61, 1.90)	0.69 (-0.57, 1.94)	0.99 (-0.24, 2.22)
Q3	0.58 (-0.65, 1.81)	0.63 (-0.61, 1.87)	1.49 (0.28, 2.71)*
Q4	1.37 (0.17, 2.56)*	1.40 (0.21, 2.60)*	2.71 (1.53, 3.89)†
p for trend	0.033	0.030	< 0.001
Stratified by gender[§]			
Boys	0.00 (-0.03, 0.03)	0.00 (-0.03, 0.03)	0.02 (-0.01, 0.05)
Girls	0.02 (0.00, 0.03)†	0.02 (0.00, 0.03)†	0.03 (0.02, 0.04)†
Stratified by race[§]			
Mexican American	0.01 (-0.01, 0.03)	0.01 (-0.01, 0.03)	0.02 (0.00, 0.04)*
Other Hispanic	0.01 (-0.02, 0.04)	0.01 (-0.02, 0.04)	0.02 (-0.01, 0.05)
Non-Hispanic white	0.01 (-0.00, 0.03)	0.01 (-0.00, 0.03)	0.03 (0.02, 0.05)†
Non-Hispanic black	0.00 (-0.02, 0.02)	0.00 (-0.02, 0.02)	0.01 (-0.01, 0.03)
Other races	0.06 (-0.00, 0.12)	0.06 (-0.00, 0.12)	0.07 (0.01, 0.13)

* $p < 0.05$, † $p < 0.01$, ‡ $p < 0.001$. Model 1 was not adjusted. Model 2 was adjusted for gender, age, and race. Model 3 was adjusted for gender, age, race, education, family income-poverty ratio, BMI, and smoking exposure. §In the subgroup analyses stratified by gender or race, the model was not adjusted for the stratification variables themselves. ALT: alanine aminotransferase; BMI: body mass index; CI: confidence interval.

Table V. Association between serum iron and AST

	Model 1, β (95 % CI)	Model 2, β (95 % CI)	Model 3, β (95 % CI)
Iron	0.01 (0.00, 0.02)†	0.01 (0.00, 0.02)†	0.01 (0.00, 0.02)†
Quartiles of iron			
Q1 (lowest quartile)	Reference	Reference	Reference
Q2	0.50 (-0.33, 1.33)	0.50 (-0.33, 1.33)	0.52 (-0.31, 1.35)
Q3	1.13 (0.32, 1.95)†	1.14 (0.32, 1.96)†	1.25 (0.43, 2.07)†
Q4	1.03 (0.24, 1.82)*	1.03 (0.24, 1.82)*	1.24 (0.43, 2.04)†
p for trend	0.005	0.005	< 0.001

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Table V (Cont.). Association between serum iron and AST

	Model 1, β (95 % CI)	Model 2, β (95 % CI)	Model 3, β (95 % CI)
Stratified by gender[§]			
Boys	0.00 (-0.02, 0.02)	0.00 (-0.02, 0.02)	0.01 (-0.02, 0.03)
Girls	0.01 (0.00, 0.02) [†]	0.01 (0.00, 0.02) [†]	0.01 (0.01, 0.02) [‡]
Stratified by race[§]			
Mexican American	0.01 (-0.00, 0.02)	0.01 (-0.00, 0.02)	0.01 (-0.00, 0.02)
Other Hispanic	0.01 (-0.01, 0.04)	0.01 (-0.01, 0.04)	0.01 (-0.02, 0.04)
Non-Hispanic white	0.02 (0.00, 0.03) [*]	0.02 (0.00, 0.03) [*]	0.02 (0.01, 0.03) [‡]
Non-Hispanic black	0.00 (-0.01, 0.01)	0.00 (-0.01, 0.01)	0.00 (-0.01, 0.02)
Other races	0.02 (-0.01, 0.05)	0.02 (-0.01, 0.05)	0.03 (-0.00, 0.06)

* $p < 0.05$, [†] $p < 0.01$, [‡] $p < 0.001$. Model 1 was not adjusted. Model 2 was adjusted for gender, age, and race. Model 3 was adjusted for gender, age, race, education, family income-poverty ratio, BMI, and smoking exposure. [§]In the subgroup analyses stratified by gender or race, the model was not adjusted for the stratification variables themselves. AST: aspartate aminotransferase; BMI: body mass index; CI: confidence interval.

Table VI. Associations between serum ferritin, iron, elevated ALT and elevated AST

	Model 1, OR (95 % CI)	Model 2, OR (95 % CI)	Model 3, OR (95 % CI)
A	1.013548 (1.013531, 1.013564) [‡]	1.013529 (1.013513, 1.013546) [‡]	1.011931 (1.011914, 1.011948) [‡]
B	1.012904 (1.012878, 1.012929) [‡]	1.012938 (1.012912, 1.012964) [‡]	1.011966 (1.011940, 1.011992) [‡]
C	1.000143 (1.000126, 1.000161) [‡]	1.000289 (1.000272, 1.000306) [‡]	1.004320 (1.004301, 1.004338) [‡]
D	1.006182 (1.006148, 1.006216) [‡]	1.005932 (1.005898, 1.005965) [‡]	1.007938 (1.007904, 1.007973) [‡]

* $p < 0.05$, [†] $p < 0.01$, [‡] $p < 0.001$. A: serum ferritin and elevated ALT. B: serum ferritin and elevated AST. C: serum iron and elevated ALT. D: serum iron and elevated AST. Elevated ALT: ALT ≥ 26 U/l in male adolescents and ≥ 22 U/l in female adolescents. Elevated AST: AST ≥ 37 U/l. Model 1 was not adjusted. Model 2 was adjusted for gender, age, and race. Model 3 was adjusted for gender, age, race, education, family income-poverty ratio, BMI, and smoking exposure. ALT: alanine aminotransferase; AST: aspartate aminotransferase; BMI: body mass index; CI: confidence interval.

SMOOTH CURVE FITTINGS AND SENSITIVITY ANALYSIS

We used smooth curve fittings to characterize the associations of serum ferritin, iron, ALT, and AST (Fig. 2). Overall, serum ferritin and iron levels were positively correlated with ALT and AST levels. However, in the relationships between ferritin and ALT and AST (Fig. 2A and B), the curve showed a slight downward slope when the ferritin concentration reached about 200 ng/ml. According to the World Health Organization (WHO) recommendations, the upper limit of normal for serum ferritin could be set to 200 ng/ml (14). Therefore, we performed a sensitivity analysis by excluding 22 subjects with serum ferritin > 200 ng/ml from the original population. Characteristics of the new population ($n = 3,382$) are shown in the supplementary table I. Through the same methods, we found that the main results remained the same basically about the associations between serum ferritin, ALT, AST, elevated ALT, and elevated AST in the sensitivity analysis (Supplementary Tables II–IV), reflecting the robust of the present results.

DISCUSSION

Since the prevalence of liver disease in adolescents has increased recently, it is necessary to explore more risk factors of

liver function and better understand the involved mechanisms to protect the liver health of adolescents. In the present study, we investigated the associations between serum ferritin, iron, and liver transaminases using NHANES data.

Recently, associations between serum ferritin levels and liver diseases have been investigated. For example, a cross-sectional study indicated that serum ferritin levels may be related to advanced liver fibrosis in treatment-naive autoimmune hepatitis (8). Yang et al. (7) found that American adults with non-alcoholic fatty liver disease had higher serum ferritin levels. Additionally, Yan et al. (15) found that serum ferritin levels were associated with non-alcoholic fatty liver disease in middle-aged and older patients with type 2 diabetes. However, it is still necessary to explore the relationship between serum ferritin and liver function in adolescents since different countries or populations may affect the results. Although a previous study, including 150 male pediatric patients, had indicated that serum ferritin levels were correlated with magnetic resonance imaging (MRI) liver fat, visceral adipose tissue content, ALT, and AST levels in male adolescents with obesity (10), the associations between serum ferritin, iron, and liver transaminases remained unclear due to the limited subjects or others. The present study included 3,404 adolescents from NHANES and indicated that serum ferritin and iron levels were positively associated with serum ALT and AST levels, as

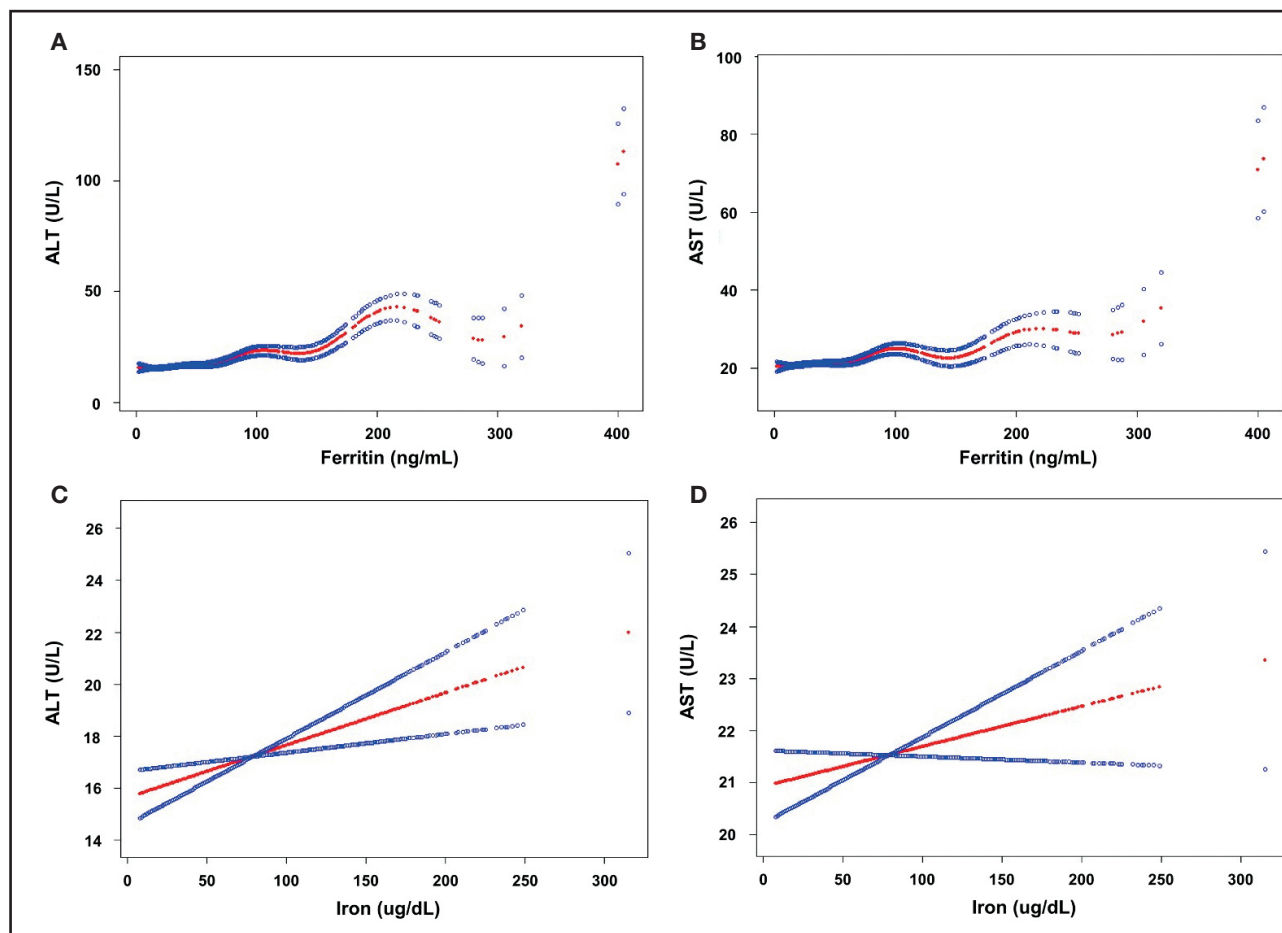


Figure 2.

Associations between serum ferritin, iron, and liver transaminases. A. Serum ferritin and ALT. B. Serum ferritin and AST. C. Serum iron and ALT. D. Serum iron and AST. Solid red line represents the smooth curve fit between variables, and blue bands represent the 95 % CI from the fit. Gender, age, race, education, family income-poverty ratio, BMI, and smoking exposure were adjusted. ALT: alanine aminotransferase; AST: aspartate aminotransferase; BMI: body mass index; CI: confidence interval.

well as the elevated ALT and elevated AST in adolescents, which provides further evidences that serum ferritin and iron may be potential biomarkers for liver damage.

A positive association was found between serum ferritin and ALT in all genders, but only remained positive in girls between serum ferritin and AST. Additionally, the positive correlations kept present among girls between serum iron and ALT or AST. These results suggest that the relationship between ferritin or iron and liver transaminases differs by sex and this relationship may be more sensitive in girls. Aigner E et al. (16) found a significant correlation of body iron stores and anthropomorphic features of the metabolic syndrome in male students, and additionally found that fasting insulin levels and insulin resistance were related to both ALT levels and soluble transferrin receptor/ferritin in female students. Other study suggested that iron overload is associated with insulin resistance in men, but not in women (17). These studies support our findings that sex may affect the association of iron status and other markers, but the mechanisms are still unknown. Notably, physiological iron status may differ between

men and women, and iron deficiency as well as iron overload affect them differently (18). Endocrine changes associated with adolescence may also represent a potential link (16). Additionally, genetic and lifestyle factors, such as dietary habits in different regions or populations, may influence the relationship between them (17). Further studies are required to investigate the pathophysiological mechanisms by which increased serum ferritin/iron levels are associated with liver transaminases in different genders.

Notably, patients with non-alcoholic fatty liver disease had higher serum ferritin level which was positively associated with liver fat, and had lower hepatic and systemic insulin sensitivity and β -cell function (19). The abovementioned studies demonstrated a hyperferritinemia in patients with various liver diseases, which may be due to hepatic inflammation and adiponectin as a marker of insulin resistance. Additionally, serum iron was commonly elevated in some liver diseases, such as non-alcoholic fatty liver disease, which is also known as metabolic abnormality iron overload syndrome (7). These phenomena may be associat-

ed with metabolic derangements or reactive metabolic inflammation. When hepatic iron deposition occurs, iron could catalyze the formation of toxic hydroxyl radicals, which mediate cellular damage (10). However, the potential mechanisms underlying these phenomena still need to be further explored.

In the present study, we have considered several possible confounding factors, including gender, age, race, education, family income-poverty ratio, BMI, and smoking exposure. However, we could not fully consider all potential confounding factors, such as alcohol drinking, since this is a retrospective study and the database has limited variables. We also could not infer a causal relationship between serum ferritin and iron levels and liver transaminases because this is a cross-sectional study. We need to further clarify their relationship and its underlying mechanisms through prospective studies and laboratory studies. Additionally, the results of this study may be highly relevant to the American adolescents because we used nationally representative samples

from NHANES. Therefore, further studies are required to investigate these associations in other populations.

CONCLUSIONS

Based on the NHANES data, it was found that serum ferritin and iron were positively correlated to ALT and AST. The association between serum ferritin and ALT remained positive in all genders and races, and remained positive in girls and several races between serum ferritin and AST. The positive correlations kept present among girls between serum iron and ALT, and also kept present among girls and non-Hispanic whites between serum iron and AST. Serum ferritin and iron levels were also positively correlated to elevated ALT and elevated AST. Further studies are required to confirm the exact relationships between serum ferritin, iron, and liver transaminases, and the underlying mechanisms.

Supplementary Table I. Characteristics of the study population in the sensitivity analysis

	All (n = 3,382)	Boys (n = 477)	Girls (n = 2,905)	p value
Age (years)	15.43 ± 2.25	15.15 ± 2.23	15.34 ± 2.28	0.099
BMI (kg/m ²)	24.47 ± 6.11	23.70 ± 5.55	23.94 ± 5.77	0.387
Ferritin (ng/ml)	40.06 ± 31.37	41.74 ± 30.70	41.19 ± 31.20	0.718
Iron (ug/dl)	78.93 ± 37.44	84.72 ± 40.61	82.57 ± 38.02	0.251
ALT (U/l)	16.99 ± 13.43	16.77 ± 13.35	16.58 ± 12.06	0.752
Elevated ALT (%)	13.42	8.07	12.54	0.005
AST (U/l)	21.43 ± 9.16	21.64 ± 9.86	21.25 ± 7.84	0.330
Elevated AST (%)	2.42	2.97	2.26	0.335
<i>Race (%)</i>				
Mexican American	26.23	18.90	26.52	< 0.001
Other Hispanic	8.96	6.17	9.05	
Non-Hispanic white	28.80	33.68	28.96	
Non-Hispanic black	24.72	16.12	24.61	
Other races	11.30	25.14	10.87	
<i>Education (%)</i>				
Less than high school	83.83	84.65	84.21	0.706
High school or equivalent	10.08	9.68	9.17	
More than high school	6.09	5.68	6.61	
<i>Family income-poverty ratio (%)</i>				
< 1	27.53	16.56	20.72	0.209
≥ 1 and < 3	39.77	38.35	36.17	
≥ 3	24.75	37.79	36.14	
Unknown	7.95	7.30	6.97	
<i>Smoking exposure (%)</i>				
Unexposed	27.79	29.75	30.04	0.883
Low-exposure	64.99	62.02	62.38	
High-exposure	7.21	8.23	7.58	

Mean ± SD for continuous variables; p values were calculated by weighted linear regression model; (%) for categorical variables; p values were calculated by weighted Chi-squared test. All p values were used for comparisons between different genders. ALT: alanine aminotransferase; AST: aspartate aminotransferase; BMI: body mass index.

Supplementary Table II. The association between serum ferritin and ALT in the sensitivity analysis

	Model 1, β (95 % CI)	Model 2, β (95 % CI)	Model 3, β (95 % CI)
Ferritin	0.07 (0.06, 0.08) [‡]	0.07 (0.06, 0.08) [‡]	0.06 (0.05, 0.08) [‡]
Quartiles of ferritin			
Q1 (lowest quartile)	Reference	Reference	Reference
Q2	-0.10 (-1.30, 1.09)	-0.11 (-1.31, 1.10)	0.43 (-0.75, 1.61)
Q3	0.75 (-0.42, 1.93)	0.79 (-0.39, 1.97)	1.03 (-0.13, 2.19)
Q4	4.24 (3.06, 5.42) [‡]	4.24 (3.05, 5.42) [‡]	4.19 (3.03, 5.36) [‡]
p for trend	< 0.001	< 0.001	< 0.001
Stratified by gender[§]			
Boys	0.04 (0.00, 0.08) [*]	0.04 (0.00, 0.08) [*]	0.04 (0.00, 0.08) [*]
Girls	0.07 (0.06, 0.09) [‡]	0.07 (0.06, 0.09) [‡]	0.07 (0.06, 0.08) [‡]
Stratified by race[§]			
Mexican American	0.08 (0.06, 0.10) [‡]	0.08 (0.06, 0.10) [‡]	0.07 (0.05, 0.10) [‡]
Other Hispanic	0.05 (0.02, 0.09) [‡]	0.05 (0.02, 0.08) [‡]	0.04 (0.01, 0.07) [‡]
Non-Hispanic white	0.08 (0.06, 0.10) [‡]	0.08 (0.06, 0.10) [‡]	0.07 (0.05, 0.09) [‡]
Non-Hispanic black	0.05 (0.02, 0.07) [‡]	0.05 (0.03, 0.07) [‡]	0.04 (0.02, 0.06) [‡]
Other races	0.10 (0.03, 0.16) [‡]	0.10 (0.03, 0.16) [‡]	0.10 (0.03, 0.17) [‡]

^{*} $p < 0.05$, [†] $p < 0.01$, [‡] $p < 0.001$. Model 1 was not adjusted. Model 2 was adjusted for gender, age, and race. Model 3 was adjusted for gender, age, race, education, family income-poverty ratio, BMI, and smoking exposure. [§]In the subgroup analyses stratified by gender or race, the model was not adjusted for the stratification variables themselves. ALT: alanine aminotransferase; BMI: body mass index; CI: confidence interval.

Supplementary Table III. The association between serum ferritin and AST in the sensitivity analysis

	Model 1, β (95 % CI)	Model 2, β (95 % CI)	Model 3, β (95 % CI)
Ferritin	0.03 (0.02, 0.04) [‡]	0.03 (0.02, 0.04) [‡]	0.03 (0.02, 0.04) [‡]
Quartiles of ferritin			
Q1 (lowest quartile)	Reference	Reference	Reference
Q2	0.07 (-0.73, 0.87)	0.03 (-0.77, 0.84)	0.06 (-0.75, 0.87)
Q3	0.80 (0.01, 1.58) [*]	0.77 (-0.02, 1.56)	0.80 (0.01, 1.59) [*]
Q4	1.84 (1.04, 2.63) [‡]	1.85 (1.05, 2.64) [‡]	1.92 (1.12, 2.71) [‡]
p for trend	< 0.001	< 0.001	< 0.001
Stratified by gender[§]			
Boys	0.02 (-0.01, 0.04)	0.02 (-0.01, 0.05)	0.02 (-0.01, 0.05)
Girls	0.03 (0.02, 0.04) [‡]	0.03 (0.02, 0.04) [‡]	0.03 (0.02, 0.04) [‡]
Stratified by race[§]			
Mexican American	0.04 (0.02, 0.05) [‡]	0.04 (0.02, 0.05) [‡]	0.04 (0.02, 0.05) [‡]
Other Hispanic	0.02 (-0.01, 0.05)	0.02 (-0.01, 0.05)	0.01 (-0.02, 0.05)
Non-Hispanic white	0.04 (0.02, 0.06) [‡]	0.04 (0.02, 0.06) [‡]	0.04 (0.02, 0.06) [‡]
Non-Hispanic black	0.01 (-0.00, 0.03)	0.01 (-0.00, 0.03)	0.01 (-0.00, 0.03)
Other races	0.02 (-0.01, 0.06)	0.02 (-0.01, 0.06)	0.03 (-0.00, 0.07)

^{*} $p < 0.05$, [†] $p < 0.01$, [‡] $p < 0.001$. Model 1 was not adjusted. Model 2 was adjusted for gender, age, and race. Model 3 was adjusted for gender, age, race, education, family income-poverty ratio, BMI, and smoking exposure. [§]In the subgroup analyses stratified by gender or race, the model was not adjusted for the stratification variables themselves. AST: aspartate aminotransferase; BMI: body mass index; CI: confidence interval.

Supplementary Table IV. The association between serum ferritin and elevated ALT and elevated AST in the sensitivity analysis

	Model 1, OR (95 % CI)	Model 2, OR (95 % CI)	Model 3, OR (95 % CI)
A	1.013448 (1.013430, 1.013467) [‡]	1.013439 (1.013420, 1.013457) [‡]	1.012073 (1.012054, 1.012092) [‡]
B	1.014734 (1.014701, 1.014768) [‡]	1.014990 (1.014957, 1.015024) [‡]	1.014230 (1.014196, 1.014264) [‡]

* $p < 0.05$, [†] $p < 0.01$, [‡] $p < 0.001$. A: serum ferritin and elevated ALT. B: serum ferritin and elevated AST. Elevated ALT: ALT ≥ 26 U/l in male adolescents and ≥ 22 U/l in female adolescents. Elevated AST: AST ≥ 37 U/l. Model 1 was not adjusted. Model 2 was adjusted for gender, age, and race. Model 3 was adjusted for gender, age, race, education, family income-poverty ratio, BMI, and smoking exposure. ALT: alanine aminotransferase; AST: aspartate aminotransferase; BMI: body mass index; CI: confidence interval.

REFERENCES

- Williams R, Ashton K, Aspinall R, Bellis MA, Bosanquet J, Cramp ME, et al. Implementation of the Lancet Standing Commission on Liver Disease in the UK. *Lancet* 2015;386(10008):2098-111. DOI: 10.1016/S0140-6736(15)00680-7
- Younossi Z, Tacke F, Arrese M, Chander Sharma B, Mostafa I, Bugianesi E, et al. Global perspectives on nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. *Hepatology* 2019;69(6):2672-82. DOI: 10.1002/hep.30251
- Tripathy R, Panda AK, Das BK. Serum ferritin level correlates with SLE-DAI scores and renal involvement in SLE. *Lupus* 2015;24(1):82-9. DOI: 10.1177/0961203314552290
- Cacoub P, Choukroun G, Cohen-Solal A, Luporsi E, Peyrin-Biroulet L, Peoc'h K, et al. Iron deficiency screening is a key issue in chronic inflammatory diseases: a call to action. *J Intern Med* 2022;292(4):542-56. DOI: 10.1111/joim.13503
- Ameka M, Hasty AH. Paying the iron price: liver iron homeostasis and metabolic disease. *Compr Physiol* 2022;12(3):3641-63. DOI: 10.1002/cphy.c210039
- Gao H, Jin Z, Bandyopadhyay G, Wang G, Zhang D, Rocha KCE, et al. Aberrant iron distribution via hepatocyte-stellate cell axis drives liver lipogenesis and fibrosis. *Cell Metab* 2022;34(8):1201-13e5. DOI: 10.1016/j.cmet.2022.07.006
- Yang N, Lu Y, Cao L, Lu M. The association between non-alcoholic fatty liver disease and serum ferritin levels in American adults. *J Clin Lab Anal* 2022;36(2):e24225. DOI: 10.1002/jcla.24225
- Chen Q, Gao M, Yang H, Mei L, Zhong R, Han P, et al. Serum ferritin levels are associated with advanced liver fibrosis in treatment-naive autoimmune hepatitis. *BMC Gastroenterol* 2022;22(1):23. DOI: 10.1186/s12876-022-02098-z
- Yi KH, Hwang JS, Lim SW, Lee JA, Kim DH, Lim JS. Ferritin level is associated with metabolic syndrome and elevated alanine aminotransferase in children and adolescents. *J Pediatr Endocrinol Metab* 2016;29(12):1337-44.
- Morwald K, Aigner E, Bergsten P, Brunner SM, Forslund A, Kullberg J, et al. Serum ferritin correlates with liver fat in male adolescents with obesity. *Front Endocrinol (Lausanne)* 2020;11:340. DOI: 10.3389/fendo.2020.00340
- Du SX, Lu LL, Geng N, Victor DW, Chen LZ, Wang C, et al. Association of serum ferritin with non-alcoholic fatty liver disease: a meta-analysis. *Lipids Health Dis* 2017;16(1):228. DOI: 10.1186/s12944-017-0613-4
- Curtin LR, Mohadjer LK, Dohrmann SM, Montaquila JM, Kruszan-Moran D, Mirel LB, et al. The National Health and Nutrition Examination Survey: sample design, 1999-2006. *Vital Health Stat* 2012;(155):1-39.
- Xu Z, Weng Z, Liang J, Liu Q, Zhang X, Xu J, et al. Association between urinary cadmium concentrations and liver function in adolescents. *Environ Sci Pollut Res Int* 2022;29(26):39768-76. DOI: 10.1007/s11356-022-18950-4
- World Health Organization (WHO). Guideline on use of ferritin concentrations to assess iron status in individuals and populations. WHO Guidelines Approved by the Guidelines Review Committee. Geneva: WHO; 2020.
- Yan JX, Pan BJ, Zhao PP, Wang LT, Liu JF, Fu SB. Serum ferritin is correlated with non-alcoholic fatty liver disease in middle-aged and older patients with type 2 diabetes. *Endocr Connect* 2021;10(12):1560-9. DOI: 10.1530/EC-21-0367
- Aigner E, Hinz C, Steiner K, Rossmann B, Pflieger J, Hohla F, et al. Iron stores, liver transaminase levels and metabolic risk in healthy teenagers. *Eur J Clin Invest* 2010;40(2):155-63. DOI: 10.1111/j.1365-2362.2009.02238.x
- Kim CH, Kim HK, Bae SJ, Park JY, Lee KU. Association of elevated serum ferritin concentration with insulin resistance and impaired glucose metabolism in Korean men and women. *Metabolism* 2011;60(3):414-20. DOI: 10.1016/j.metabol.2010.03.007
- Li X, Duan X, Tan D, Zhang B, Xu A, Qiu N, et al. Iron deficiency and overload in men and woman of reproductive age, and pregnant women. *Reprod Toxicol* 2023;118:108381. DOI: 10.1016/j.reprotox.2023.108381
- Utzschneider KM, Largajolli A, Bertoldo A, Marcovina S, Nelson JE, Yeh MM, et al. Serum ferritin is associated with non-alcoholic fatty liver disease and decreased Beta-cell function in non-diabetic men and women. *J Diabetes Complications* 2014;28(2):177-84. DOI: 10.1016/j.jdiacomp.2013.11.007