Useful parameters for distinguishing nonalcoholic steatohepatitis with mild steatosis from cryptogenic chronic hepatitis in the Japanese population

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Running title: Predictors of NASH with mild steatosis

Abstract

Background/Aims: Since detecting mild steatosis is difficult by abdominal ultrasonography (US), nonalcoholic steatohepatitis (NASH) with mild steatosis may sometimes be confused with cryptogenic chronic hepatitis. We aimed to test this possibility and to isolate factors that may indicate NASH.

Methods: First, 53 Japanese patients diagnosed as having cryptogenic chronic hepatitis by laboratory examination and US were enrolled. These patients were histologically divided into NASH and non-NASH groups, and their clinical features were compared. Second, the diagnostic accuracy of predictors of NASH was examined prospectively.

Results: Fifteen patients (28%) were histologically diagnosed as having NASH with mild steatosis. Multivariable analysis revealed that body mass index (BMI) and serum ferritin level were independent predictors of NASH. The best cutoff values to detect NASH were assessed by using receiver operating characteristic curves: BMI > 25.2 kg/m^2 and serum ferritin level > 142 ng/mL. When both markers were concomitantly negative, the negative predictive value to detect NASH was 100%.

Conclusions: In cases of mild steatosis, US is not a perfect tool for the accurate diagnosis of NASH. BMI and serum ferritin level are useful discriminators of NASH from cryptogenic chronic hepatitis, and might be helpful markers for diagnosing NASH more accurately in Japanese patients.

Chronic hepatitis is histologically characterized by sustained hepatocyte injury with apparent inflammation, and is clinically defined as the persistent elevation of serum aminotransferase levels for more than 6 months. The common causes of chronic hepatitis include persistent viral infection such as that of hepatitis B virus (HBV) and hepatitis C virus (HCV), autoimmune disorders, and intake of alcohol, drugs, or chemicals. However, patients with cryptogenic chronic hepatitis or unexplained elevation of serum aminotransferase levels still exist whose exact pathogenesis has not been verified. Because cryptogenic chronic hepatitis may progress to cirrhosis, accurate diagnosis and treatment is needed.

Nonalcoholic steatohepatitis (NASH) is defined as a disease entity showing characteristic pathological findings common to alcoholic liver disease, including hepatic steatosis, hepatocellular ballooning, and perisinusoidal/pericellular fibrosis, despite no alcohol consumption. Skelly et al. have reported that 34% of British patients with unexplained abnormal liver function tests are later diagnosed as NASH by liver biopsy (1), and in the USA, most patients with unexplained aminotransferase elevation are considered to have nonalcoholic fatty liver disease (NAFLD) (2, 3). Recently, NASH has also been recognized as one of the major causes of chronic hepatitis in Japan, though its prevalence in patients with unexplained aminotransferase elevation or cryptogenic chronic hepatitis remains unclear.

Radiological imaging devices such as ultrasonography (US), computed tomography, and magnetic resonance are indispensable in evaluating hepatic steatosis. When lipid accumulation is observed in more than 33% of hepatocytes, these modalities have a strong ability to accurately diagnose hepatic steatosis (4). Of these, US is the least invasive and thus most preferred method. If fatty infiltration is evident by US examination, it is simple to conclude that unexplained persistent elevation of aminotransferase levels may be caused by NAFLD or NASH. However, mild (accumulation of triglycerides in less than 33% of hepatocytes) or focal steatosis can be underestimated by imaging modalities such as US (5). Additionally, in advanced stages of NASH, it is increasingly difficult to detect hepatic steatosis by imaging modalities since steatosis regresses and becomes focal as fibrosis progresses. Therefore, NASH with mild steatosis or advanced fibrosis may be confused with cryptogenic chronic hepatitis.

Based on these premises, we hypothesized that NASH presenting atypical features such as mild steatosis may be erroneously included with cryptogenic chronic hepatitis, and we retrospectively examined histological findings in patients who were diagnosed as having cryptogenic chronic hepatitis based on biochemical data and abdominal US to re-evaluate the prevalence of NASH. We also compared the clinical features between NASH and non-NASH (intrinsically cryptogenic chronic hepatitis) groups and sought to find useful markers to differentiate NASH from cryptogenic chronic hepatitis that can be used in addition to US.

Patients and methods
Study 1
Patients

Chronic hepatitis was clinically defined by the following criteria: (1) elevation of serum aminotransferase levels (> 40 IU/L) on two or more occasions during a period of at least 6 months and (2) exclusion of extrahepatic-origin elevation of serum aminotransferase levels such as myopathy and thyroid diseases by measuring serum levels of lactate dehydrogenase, creatine kinase, thyroid hormones, and thyroid-stimulating hormone. Patients who showed evidence as having liver cirrhosis were excluded. One-thousand six-hundred ninety-one patients with chronic hepatitis who underwent liver biopsy at Shinshu University Hospital or affiliated hospitals between April 1, 1990 and September 30, 2004 were enrolled in this study. The diagnosis of cryptogenic chronic hepatitis was made according to the exclusion criteria shown in Fig. 1: (1) no consumption of alcohol; (2) negative results for hepatitis B surface antigen (HBsAg), high titer of hepatitis B core antibody (anti-HBc), and anti-HCV antibody (anti-HCV); (3) exclusion of other liver diseases such as drug-induced liver injury, autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, Wilson's disease, hereditary hemochromatosis, and α1-antitrypsin deficiency; and (4) exclusion of obvious hepatic steatosis in abdominal US, that is, positive hepatorenal contrast and blurring of vascular wall and/or profound attenuation of the diaphragm. Patients presenting obvious hepatic steatosis were excluded from this study, since typical NAFLD or NASH is easily distinguishable using US. In total, 53 Japanese patients were clinically diagnosed as having cryptogenic chronic hepatitis (Fig. 1).

At the time of admission for liver biopsy, body mass index (BMI) was calculated. Patients were considered to have hypertension if their systolic/diastolic pressure was greater than 140/90 mmHg, or if they were taking anti-hypertensive drugs. Patients were considered to be diabetic if they had a fasting glucose level equal to or higher than 126 mg/dL, or if they were taking insulin or oral hypoglycemic drugs. Patients were considered to have hyperlipidemia if their fasting serum levels of cholesterol and triglycerides were equal to or higher than 220 mg/dL and 150 mg/dL, respectively, or if they were taking lipid-lowering drugs.

Laboratory examination

All data were obtained in a fasting state and measured by standard methods. The homeostasis model assessment for insulin resistance (HOMA-IR) was calculated using the following equation: [fasting glucose (mg/dL) x fasting insulin (μU/mL)]/405. A HOMA-IR greater than 2.0 is considered to indicate the presence of insulin resistance. If the patient had a fasting glucose level equal to or higher than 140 mg/dL, or was taking insulin, HOMA-IR was not calculated.

Histological diagnosis of NASH

Before liver biopsy, informed consent was obtained from each patient. Liver biopsy specimens were immediately fixed in 10% neutral formalin. Sections were cut at 4-μm thickness and stained with hematoxylin and eosin and Azan-Mallory methods. Histological diagnosis of NASH was made according to the following criteria: macrovesicular steatosis mainly present in zone 3, hepatocellular ballooning and/or perisinusoidal/pericellular fibrosis. Histological findings were classified using the grading/staging system proposed by Brunt et al. (6) with minor modifications. The degree of hepatic steatosis was expressed as the percentage of steatotic hepatocytes in biopsied specimens. The appearance frequency of hepatocellular ballooning, glycogenated nuclei, and eosinophilic intracytoplasmic inclusion bodies was graded as absent, few, or many based on the number of hepatocytes showing the respective changes. The activity of lobular and portal inflammation was graded as absent, mild, moderate, or severe. Perisinusoidal/pericellular fibrosis was scored as absent, mild, or severe based on the proportion of zone 3 area involved, and portal fibrosis was assessed

as absent, periportal, bridging, or cirrhosis. Histological diagnosis was made be three experts (ET, NT, KY).

Detection of hepatic steatosis by US

Each patient underwent abdominal US (Hitachi model EUB-525 equipped with a 3.5 MHz convex-type transducer, Hitachi, Japan) in a fasting state. The presence of hepatic steatosis was assessed independently by three hepatologists according to findings such as hepatorenal contrast, blurring of the vascular wall, and profound attenuation of the diaphragm.

Study 2

To estimate the diagnostic accuracy of the markers found in Study 1, 256 patients with elevated serum aminotransferase levels who underwent liver biopsy between October 1, 2004 and March 31, 2006 were eligible for entry into this prospective study. Exclusion criteria were positive for HBsAg, anti-HBc, anti-HCV, anti-mitochondrial antibody, the presence of the history of alcohol comsumption or hepatotoxic drug intake, and the presence of hepatic steatosis, which was easily detectable by US. The remaining 22 patients were divided into 3 groups according to the number of positive markers, and the final diagnosis was performed histologically. Diagnostic accuracy was calculated by sensitivity, specificity, and positive and negative predictive values (PPV and NPV, respectively).

Statistical analysis

Statistical analyses were performed using SPSS software 11.5J for Windows (SPSS Inc., Chicago, Illinois). Comparison between the groups was made using Fisher's exact probability test for the categorical variables, χ^2 test for the histological scores, and Mann-Whitney U-test for the continuous variables, respectively. To assess the use of clinical parameters in differentiating NASH from cryptogenic chronic hepatitis, we constructed receiver operating characteristic (ROC) curves by plotting the sensitivity against the reverse specificity (1 minus specificity) for each value. In this assessment, a larger area under the ROC curve (AUC) corresponds to a more useful marker for diagnosing NASH. The most appropriate cutoff point for the diagnosis of NASH was

the point at which the sum of the sensitivity and the specificity was maximized. To identify independent predictors of NASH, multivariable logistic regression analysis was conducted. A probability value of less than 0.05 was considered statistically significant.

Results

Study 1

NASH in cryptogenic chronic hepatitis

In this study, we addressed the specific group of subjects with unexplained aminotransferase elevation and normal US findings. Of the 53 patients who were clinically diagnosed as having cryptogenic chronic hepatitis, 15 (28%) fulfilled the histological diagnostic criteria of NASH (Fig. 1). The histological features of these 15 patients, who were not diagnosed as having steatosis by US, but were histologically confirmed as having NASH, are shown in Table 1. The degree of hepatic steatosis was generally mild (<33% of hepatocytes in the biopsy involved), so steatosis was hard to detect by US (Figs. 2 and 3). Ballooned hepatocytes, glycogenated nuclei, and perisinusoidal/pericellular fibrosis in zone 3 were observed in all NASH patients, and eosinophilic intracytoplasmic inclusion bodies were detected in 60% of these patients. Lobular and portal inflammation was relatively mild, and portal fibrosis was variable. These results suggest that detection of NASH with mild steatosis is clinically unreliable using US only.

Comparison of histological findings between biopsy-proven NASH patients and non-NASH patients

We compared the histological findings between the biopsy-proven NASH patients, i.e. patients having NASH with mild steatosis (NASH group, n = 15), and non-NASH patients, i.e. patients having intrinsically cryptogenic chronic hepatitis (non-NASH group, n = 38). As shown in Table 1, the prevalence of macrovesicular steatosis, hepatocellular ballooning, glycogenated nuclei, eosinophilic intracytoplasmic inclusion bodies, and perisinusoidal/pericellular fibrosis was significantly higher in the NASH group. Three patients in the non-NASH group (3 in 38 patients, 8%) exhibited mild steatosis but could not be diagnosed as having NASH because of the absence of ballooned hepatocytes or perisinusoidal/pericellular fibrosis. Although the activity of

lobular inflammation was similar in both groups, portal inflammation tended to be more severe in the non-NASH group. In NASH livers, lymphocytes and/or polymorphonuclear leukocytes were present in the inflammatory foci, whereas in non-NASH livers, infiltration of plasma cells and/or eosinophils, as well as lymphocytes, was observed. These results demonstrate clear histological differences between the two groups.

Comparison of clinical features between the NASH group and the non-NASH group

To explore other helpful markers for differentiating NASH with mild steatosis from cryptogenic chronic hepatitis, we compared the clinical features and laboratory findings between the two groups. As shown in Table 2 1, the prevalence of hyperlipidemia (P = 0.002), BMI (P = 0.001), fasting glucose level (P = 0.021), HOMA-IR (P = 0.009), and serum ferritin level (P = 0.001) was all significantly higher in the NASH group. The prevalence of diabetes and hypertension, serum levels of high-sensitivity C-reactive protein, aminotransferases, and γ-glutamyltransferase, immunoglobulin G and A concentrations, and hemoglobin A1c value were not significantly different between the two groups.

Multivariable analysis

Multivariable logistic regression analysis revealed that BMI and serum ferritin level were independent factors associated with NASH. The Odd ratio for BMI was 1.836 [95% confidence interval (CI), 1.063-3.173; P = 0.029), and that for serum ferritin level was 1.014 (95% CI, 1.000-1.027; P = 0.048).

ROC curve analysis

ROC curves were constructed for these two parameters. The AUCs for BMI and serum ferritin level were as great as 0.791 (95% CI, 0.644-0.938; P = 0.001) and 0.782 (95% CI, 0.651-0.914; P = 0.001), respectively. We next determined the cutoff values of these parameters for the discrimination between NASH with mild steatosis and cryptogenic chronic hepatitis by using the ROC curve. The most appropriate cutoff values were identified as BMI > 25.2 kg/m^2 and serum ferritin level > 142 ng/mL, respectively.

Study 2

We prospectively examined the diagnostic accuracy of the predictors of NASH found in Study 1. In 22 patients with persistent unexplained elevation of serum aminotransferase levels, 8 were histologically diagnosed as having NASH (Fig. 4). Most of these NASH patients exhibited severe fibrosis or cirrhosis. When both parameters were concomitantly positive, the sensitivity, specificity, and PPV and NPV to detect NASH were 87.5%, 85.7%, 77.8%, and 92.3%, respectively. On the other hand, when both parameters were concomitantly negative, the sensitivity, specificity, and PPV and NPV were 100%, 71.4%, 71.4%, and 100%, respectively. Although the number of patients enrolled in Study 2 was very limited, these results might suggest the relevance of these parameters as supporting diagnostic markers of NASH for patients with unexplained persistent aminotransferase elevation.

Discussion

The present study demonstrates that sole reliance on abdominal US might overlook NASH with mild steatosis, and that BMI and serum ferritin level are helpful for differentiating NASH with mild steatosis from cryptogenic chronic hepatitis and for diagnosing NASH more accurately.

In NASH, appropriate correction of life style and pharmacological interventions (e.g., insulin sensitizers) can reduced disease activity and prevent the progression (7, 8). These therapeutic strategies are fundamentally distinct from other types of chronic hepatitis, so accurate diagnosis is very important for NASH. US is the most common diagnostic tool for detecting hepatic steatosis, which is an essential component of NASH. Generally speaking, US has a relatively high sensitivity and specificity for the detection of hepatic steatosis. In cases with more than 33% fatty infiltration, the sensitivity and PPV of US are 100% and 62%, respectively (4). However, the diagnostic accuracy of US declines sharply in cases of less than 30% fatty infiltration. This change may be associated not only with mild or focal fatty deposition in livers, but also with inter-observer differences in image readings. Although US has been used for the diagnosis of NAFLD in several studies (9-11), there is a possibility that NASH with mild steatosis has been overlooked. Indeed, Hamaguchi et al. have described that US may lead to an incorrect diagnosis of NAFLD in 10% to 30% of cases analyzed (11).

We found that, regardless of no detection of steatosis by US, 28% of Japanese patients with unexplained elevation of serum aminotransferase levels have NASH with mild steatosis, suggesting the inadequacy of US to detect certain types of NASH. Therefore, US cannot be considered as a gold standard test for the accurate diagnosis of NASH with mild steatosis, and novel diagnostic markers of NASH, which may compensate for this imperfection, are clearly needed.

We found that BMI and serum ferritin level are highly predictive of NASH. It is well known that NASH is strongly associated with obesity and visceral fat accumulation (11-13). It has also been reported that serum ferritin level, a major determinant of NAFLD in apparently healthy obese individuals (10), is significantly correlated with the amount of visceral fat mass and hepatic steatosis (14). Furthermore, serum ferritin level has been reported to be significantly higher in NASH than that in simple steatosis, which may reflect increased hepatic iron overload and enhanced oxidative stress (15). Therefore, assessment of these markers may be useful not only for discriminating NASH from chronic hepatitis caused by unknown hepatotoxic factors, but also for diagnosing NASH more accurately and efficiently.

As shown in Fig. 3, in patients with NASH in advanced fibrosis or cirrhosis, the accuracy of hepatic steatosis detection is markedly reduced, so the correct diagnosis of NASH becomes increasingly difficult. The results obtained from Study 2 (Fig. 4) suggest that a combination of BMI and serum ferritin level might be helpful parameters for distinguishing NASH from cryptogenic chronic hepatitis, even in advanced stages. Thus, it might be possible to use these markers for the purpose to isolate NASH-derived cirrhosis (burned-out NASH) from intrinsically cryptogenic cirrhosis. Further study is needed to determine whether these markers would be really helpful for identifying the etiology in patients with cryptogenic hepatitis with advanced fibrosis or cirrhosis.

In this study, we could not assess the etiology of the difference between NASH and NASH with mild steatosis. The development of hepatic steatosis depends primarily on the changes of 3 pathways in fatty acid metabolism: increased influx of circulating nonesterified fatty acids into hepatocytes, increased de novo lipogenesis in hepatocytes, and decreased degradation through the mitochondrial β-oxidation system (16). Thus, an imbalance of these pathways might contribute to the difference in the severity of steatosis in NASH.

This study has several limitations. First, the number of patients analyzed was limited in this retrospective study, so a large-scale prospective analysis is needed to confirm these results. Second, although it has been reported that several biomarkers such as adipocytokines (e.g., adiponectin) (17), proinflammatory cytokines (e.g., tumor necrosis factor-α) (18), and oxidative stress markers (e.g., thioredoxin) (15) might be useful predictors of NASH, we could not examine these parameters in this study. In addition to BMI and serum ferritin level, measuring these biomarkers may enable us to more accurately diagnose NASH with mild steatosis. Finally, it is possible that some NASH patients might have been misplaced into the non-NASH group because of biopsy sampling errors. NASH livers show more heterogeneous histological findings than those with chronic hepatitis C (19). Moreover, the sampling variability is more significant in livers of burned-out NASH, so patients with advanced stages of NASH would be classified into the non-NASH group. Indeed, in this study, one obese female patient with hyperlipidemia, hypertension, diabetes, and hyperferritinemia was histologically diagnosed as having cryptogenic cirrhosis because of a lack of histological findings specific to NASH, but her aminotransferase levels normalized only by weight reduction, suggesting a strong likelihood of burned-out NASH. Repeated US-guided biopsy or laparoscopy-assisted biopsy (20) may minimize the possibility of sampling error in patients strongly suspicious of having NASH. To overcome these limitations, it will be mandatory to establish novel biochemical markers of NASH, which are available even in advanced stages of NASH and are independent of the amount of hepatic steatosis.

In conclusion, US is not a perfect tool for the accurate diagnosis of NASH with mild steatosis. Additionally, BMI > 25.2 kg/m^2 and serum ferritin level > 142 ng/mL may be good non-US discriminators of NASH from chronic hepatitis of unknown etiology.

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Figure Legends

Fig. 1. The number of patients with chronic hepatitis enrolled in Study 1.

The number of patients is indicated in parentheses. ALD, alcoholic liver disease; CH-C, chronic hepatitis C; CH-B, chronic hepatitis B; AIH, autoimmune hepatitis; PSC, primary sclerosing cholangitis; NAFLD, nonalcoholic fatty liver disease; US, ultrasonography.

Fig. 2. Ultrasonographic and histological findings of the NASH patient, who were clinically diagnosed as having cryptogenic hepatitis.

(A)   A 61-year-old woman with obesity and hyperlipidemia was diagnosed as having cryptogenic chronic hepatitis because of no obvious steatosis by US.

(B)   Histological evaluation confirmed the presence of mild macrovesicular steatosis, ballooned hepatocytes with eosiniphilic intracytoplasmic inclusion bodies, and mild perisinusoidal/pericellular fibrosis (upper photograph, Azan-Mallory staining, x 100; lower photograph, hematoxylin and eosin staining, x 400).

Fig. 3. Ultrasonographic and histological findings of another NASH patient.

(A)   A 72-year-old woman with diabetes was diagnosed as having cryptogenic chronic hepatitis because obvious fatty infiltration was not confirmed by US.

(B)   Histologically, mild and focal macrovesicular steatosis, hepatocellular ballooning, and advanced fibrosis were confirmed (upper photograph, Azan-Mallory staining, x 50; lower photograph, hematoxylin and eosin staining, x 400).

Fig. 4. Flow Chart in Study 2.

Twenty-two patients with persistent unexplained elevation of aminotransferase levels were enrolled in this prospective study. The number of patients is indicated in parentheses. 2+, BMI >25.2 kg/m^2 and serum ferrritin level >142 ng/mL; 1+, BMI >25.2 kg/m^2 or serum ferrritin level >142 ng/mL; 0, BMI <25.2 kg/m^2 and serum ferrritin level <142 ng/mL.

Table 1. Comparison of histological findings between NASH and non-NASH groups

	NASH (n = 15)		non-NASH (n = 38)		P
Macrovesicular steatosis					0.000
<5%	0	(0%)	35	(92%)	
5%-20%	3	(20%)	2	(5%)	
21%-33%	12	(80%)	1	(3%)	
>33%	0	(0%)	0	(0%)	
Ballooning					0.000
Absent	0	(0%)	37	(97%)	
Few	4	(27%)	1	(3%)	
Many	11	(73%)	0	(0%)	
Glycogenated nuclei					0.000
Absent	0	(0%)	33	(87%)	
Few	9	(60%)	5	(13%)	
Many	6	(40%)	0	(0%)	
Eosinophilic intracytoplasmic inclusion bodies					0.000
Absent	6	(40%)	38	(100%)	
Few	6	(40%)	0	(0%)	
Many	3	(20%)	0	(0%)	
Lobular inflammation					0.627
Absent	2	(13%)	10	(26%)	
Mild	10	(67%)	19	(50%)	
Moderate	3	(20%)	8	(21%)	
Severe	0	(0%)	1	(3%)	
Portal inflammation					0.021
Absent	4	(27%)	2	(5%)	
Mild	9	(60%)	16	(42%)	
Moderate	2	(13%)	11	(29%)	
Severe	0	(0%)	9	(24%)	
Perisinusoidal/pericellular fibrosis					0.000
Absent	0	(0%)	37	(97%)	
Mild	11	(73%)	1	(3%)	
Severe	4	(27%)	0	(0%)	
Portal fibrosis					0.508
Absent	8	(53%)	25	(66%)	
Periportal	2	(13%)	7	(18%)	
Bridging	4	(27%)	4	(11%)	
Cirrhosis	1	(7%)	2	(5%)	

Data are the number positive and prevalence (in parentheses). A P value was calculated using the χ^2 test.

Table 2. Comparison of clinical features and biochemical markers between NASH and non-NASH groups

	NASH (n = 15)		non-NASH (n = 38)		P
Age (years)	59	(39-77)	56	(14-72)	0.431
Female	57%		85%		0.532
Diabetes	27%		16%		0.143
Hyperlipidemia	47%		5%		0.002
Hypertension	27%		18%		0.275
BMI (kg/m^2)	26.9	(19.7-33.0)	23.1	(17.9-31.6)	0.001
Platelet (x10^4/μL)	18.2	(12.5-35.0)	19.9	(7.9-35.7)	0.552
hsCRP (mg/dL)	0.099	(0.017-0.496)	0.078	(0.004-0.500)	0.103
Albumin (g/dL)	4.5	(3.7-4.9)	4.3	(3.3-4.9)	0.232
AST (IU/L)	64	(19-161)	57	(16-639)	0.809
ALT (IU/L)	75	(30-238)	79	(22-604)	0.713
γGT (IU/L)	51	(37-213)	71	(9-269)	0.742
Total cholesterol (mg/dL)	213	(155-278)	178	(129-353)	0.054
Triglycerides (mg/dL)	122	(66-398)	97	(49-266)	0.088
HDL-cholesterol (mg/dL)	40	(20-61)	40	(21-74)	0.826
Immunoglobulin G (mg/dL)	1368	(677-3012)	1341	(987-2612)	0.750
Immunoglobulin A (mg/dL)	250	(99-487)	255	(80-713)	0.951
Glucose (mg/dL)	96	(77-164)	89	(56-113)	0.021
Hemoglobin A1c (%)	5.8	(5.0-7.8)	5.3	(4.4-7.2)	0.064
HOMA-IR*	2.4	(0.3-8.9)	1.2	(0.2-7.6)	0.009
Iron (μg/dL)	145	(67-325)	102	(20-266)	0.104
Transferrin saturation (%)	44	(29-94)	34	(5-90)	0.076
Ferritin (ng/mL)	229	(62-776)	120	(3-376)	0.001

Qualitative data are expressed as percentages, and quantitative data are written as medians and ranges (in parentheses). A P value for qualitative and quantitative data was calculated using Fisher's exact probability test and Mann-Whitney U-test, respectively. * HOMA-IR was not evaluated in patients receiving insulin therapy or having a fasting glucose level equal to or higher than 140 mg/dL and γGT, γ-glutamyltransferase; HDL, high density lipoprotein; HOMA-IR, homeostasis model assessment for insulin resistance.