Original Article

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Non-Alcoholic Fatty Liver Disease with Abnormal ALT Level : Role of Insulin Resistance in Predicting Liver Histology

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ABSTRACT

Background: Nonalcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver injury. Insulin resistance is central to the pathogenesis of NAFLD, and recent data indicate that non-alcoholic steatohepatitis (NASH) should be considered the hepatic manifestation of the insulin resistance syndrome.

Objective: The aim of this study was to find out if insulin resistance level can predict liver histology in NAFLD and NASH patients.

Method: One-hundred-sixty-four patients with a presumptive diagnosis of presumed NAFLD were enrolled in the study. The diagnosis of presumed NAFLD was made if liver ultrasonographic findings were compatible with hepatic steatosis and other causes of hepatitis were excluded. A presumed NAFLD patient with persistent increase of aminotransferase level of at least 1.5 times the upper normal limit for two consecutive times in 3 months, and with adequate control of the underlying disease and the body weight would be subjected to liver biopsy. Body mass index (BMI), waist circumference, fasting blood sugar, liver function test, lipid profile, insulin level, ferritin and hs-CRP were measured.

Result: Of 164 patients, 103 (63.0%) patients fulfilled the criteria for liver biopsy. Eighty-five patients (85/103; 82.5%) had insulin resistance (HOMA-IR ≥2). Only 30 patients (29.1%) gave informed consent for liver biopsy. Twenty-three patients (76.7%) had histology compatible with nonalcoholic steatohepatitis (NASH), and 7 (23.3%) patients had histology compatible with fatty liver. Six patients (85.7%) in fatty liver group and 22 patients (95.7%) in NASH group had insulin resistance. HOMA-IR of NASH patients was 4.0 ± 1.5 and of fatty liver patients was 3.9 ± 1.4, (p = 0.917).

Conclusion: HOMA-IR was not difference statistically between NASH patients and fatty liver patients.

Key words : HOMA-IR, NASH, NAFLD, fatty liver

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BACKGROUND

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver injury[1,2]. The spectrum of NAFLD encompasses simple fatty liver (steatosis), which is generally nonprogressive[3], and nonalcoholic steatohepatitis (NASH), which can lead to liver cirrhosis and liver failure[4,5].

The prognosis of NAFLD is depended on the degree of liver dysfunction and the stage of the disease. The degree of liver dysfunction is reflected from serum albumin, bilirubin and prothrombin time, but these parameters usually remain unchanged until a late stage. The stage of liver disease depends on the degree of liver fibrosis. Liver biopsy is the standard reference of the severity of fibrosis. However, liver biopsy carries limitations such as interobserver variation, sampling error, cost and potential serious complication including hemorrhage or death[6]. Thus, there is a recognized need for less-invasive strategies to identify the minority of NAFLD patients with liver fibrosis.

Insulin resistance is central to the pathogenesis of NAFLD[7,8], Recent data indicate that NASH should be considered the hepatic manifestation of the insulin resistance syndrome[8,9-11]. NAFLD accounts for the risk of advanced liver disease as observed in these patients[12,13], in addition to the well-established cardiovascular risk[14-16].

The aim of this study was to find out if insulin resistance level can predict liver histology in NAFLD and NASH patients.

PATIENTS AND METHODS

Patients

One-hundred-and-sixty-four patients with a diagnosis of presumed NAFLD were enrolled in this study. The diagnosis of NAFLD was based on hepatic ultrasonographic features compatible with hepatic steatosis ("bright" liver with increased echogenicity of the liver parenchyma compared to the renal cortex, or inability to identify intra-hepatic vessels). The exclusions were other causes of liver disease, including alcohol-induced liver disease (history of alcohol consumption of over 20 gm/day in men and 10gm/day in women, confirmed by at least one family member), drug-induced liver disease (corticosteroid, tamoxifen, amiodarone, metrotrexate, estrogen, chloroquin or penhexilene maleate), viral hepatitis, pregnancy, malignancy, previous gastrointestinal bypass surgery, and presence of other liver disease (primary biliary cirrhosis, autoimmune hepatitis, Wilson's disease, chronic viral hepatitis B or C and hereditary hemochromatosis).

A presumed NAFLD patient with persistent elevation of aminotransferase level (serum ALT ≥1.5 times the upper normal limit) for at least 2 consecutive occasions in 3 months, despite adequate control of the underlying disease and good body weight control, patient would was proceed to liver biopsy. The exclusions for liver biopsy procedure were coagulopathy (INR >1.5), thrombocytopenia (platelet ≤100,000/mL), and HIV infection.

METHODS

Anthropometric assessment

Body weight, height and waist circumference (at the highest point of the iliac crest) were determined. Body mass index (BMI) was calculated as weight (kg)/ height (m^2).

Biochemical Data

Blood samples were collected after an overnight fast at 3-month of follow up or one week before liver biopsy. Laboratory tests include complete blood count, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, alkaline phosphatase (ALP), prothrombin time (PT), INR, fasting glucose, total cholesterol, triglyceride, low density lipoprotein (LDL), high density lipoprotein (HDL), creatinine (Cr), insulin level , ferritin, and hs-CRP.

Insulin resistance was defined as HOMA-IR ≥2 which calculated from the formula: fasting serum insulin (μIU/mL) multiplied by fasting serum glucose (mmol/L), divided by 22.5[17].

Histological Data

All liver biopsy samples were reviewed by a pathologist who was blinded for the patients' data. Biopsy specimens were fixed in 10% neutralized formaldehyde, embedded in paraffin, and sections were stained with hematoxylin-eosin and Masson's trichrome. The diagnosis of NASH was based on the Brunt criteria[18]. The stage of fibrosis was scoring based on a five-point scale: stage 0, absence of fibrosis; stage 1, perisinusoidal or portal fibrosis; stage 2, perisinusoidal and portal/periportal fibrosis; stage 3, septal or bridging fibrosis; and stage 4, cirrhosis. The

advanced fibrosis defined as score ≥ F3 fibrosis. The histologic staging of NAFLD/NASH by Matteoni's Classification[19]: Class 1: simple steatosis without inflammation or fibrosis; Class 2: steatosis with lobular inflammation but without fibrosis; Class 3: additional presence of ballooned hepatocytes; Class 4: presence of either Mallory's hyaline or fibrosis. The NASH patients were defined as class 3 and class 4.

Statistical analysis

SPSS (11.5) statistical software for Windows was used to collect data, which were expressed as the mean ± SD for continuous variables. Student's *t*-test for unpaired data was used for the comparison of mean values. Group comparisons were performed by use of analysis of variance (ANOVA). Proportions and categorical variables were tested by the χ^2-test and Fisher's exact method. A *p*-value <0.05 was considered to indicate statistical significance.

RESULT

A total of 164 patients were enrolled in this study. Clinical and biochemical data of all patients are shown in Table 1. The mean BMI was 27.2 ± 4.1 kg/m^2. Sixty-eight (41.5%) patients were diagnosed as having the metabolic syndrome. The 119 patients (72.6%) had insulin resistance (HOMA-IR ≥2), and 42 patients (25.6%) were diabetic.

One-hundred-and-three patients (64.8%) had persistent elevation of aminotransferase level ≥1.5 times the upper normal limit for 2 consecutive accessions in 3 months. However, only 30 patients (29%) agreed to undergo liver biopsy.

Table 2 displays characteristics of biopsied and non-biopsied NAFLD patients with elevated serum ALT (≥1.5 times). There were no differences between the two groups regarding age, BMI, gender, underlying disease and biochemical variables. The mean age of patients in the biopsy group was 49.0 ± 8.5, 20 (66.7%) were male and 10 (33.3%) female. The mean BMI and the mean waist circumference were 27.3 ± 4.2 kg/m^2 and 88.0 ± 10 cm. Six patients (20%) had diabetes and 12 (41.4%) patients had metabolic syndrome. The mean HOMA-IR was 5.0 ± 4.2, and 28 (93.3%) patients had insulin resistance. The mean hs-CRP was 3.6 ± 4.7 and the mean serum ferritin was 336.9 ± 258.4 (ng/mL). The mean serum ALT in patients who underwent liver biopsy was not higher than those who did not done (128.3 ± 4.8 vs. 88.3 ± 3.6 IU/mL, *p* = 0.940).

Table 1. Baseline characteristic of patients with presumed NAFLD.

Variables	n = 164
Age (yrs)	52.0 ± 10.3
M/F (%)	80 (48.8) / 84 (51.2)
BMI (kg/m^2)	27.2 ± 4.1
Waist circumference (cm)	85.0 ± 82.5
History of diabetes (%)	42 (25.6)
History of hypertension (%)	77 (47.0)
History of dyslipidemia (%)	86 (52.4)
TG (mg/dL)	195.5 ± 109.1
HDL (mg/dL)	40.9 ± 9.8
FBS (mg/dL)	116.8 ± 30.2
Metabolic syndrome (%)	68 (41.5)
Insulin resistant (HOMA-IR ≥ 2)	119 (72.6)
HOMA-IR	4.4 ± 4.3
Ferritin (ng/mL)	320.1 ± 366
hs-CRP (mg/L)	3.18 ± 5.0
ALT ≥ 1.5 times	103 (64.8)
ALT (U/L)	91.0 ± 90.0

mean ± SD

Abbreviation: *BMI; body mass index, HDL; high density lipoprotein, TG; triglyceride, HDL; high density lipoprotein, FBS; fasting blood sugar, ALT; alanine aminotransferase.*

In the biopsy group, 23 patients (76.7%) had the histology compatible with nonalcoholic steatohepatitis and 7 patients (23.3%) had histology compatible with fatty liver. According to fibrosis score (F), 11 patients (36.7%) had F1 fibrosis, 10 patients (33.3%) had F2 fibrosis, 8 patients (26.7%) had F3 fibrosis, and one patients (3.3%) had F4 (cirrhosis).

Clinical and biochemical variables of fatty liver patients and NASH patients were shown in Table 3. No significant difference was detected. Mean age of NASH and fatty liver group was comparable (49.3 ± 7.7 vs. 48.3 ± 8.9). HOMA-IR in NASH patients was not higher than in fatty liver patients (4.0 ± 1.5 vs. 3.9 ± 1.4, *p* = 0.917). Mean serum ferritin in fatty liver patients was lower than in NASH patients (169.2 ± 96.0 ng/mL vs. 361.7 ± 263.2 ng/mL, *p* = 0.107). Mean serum alanine aminotransferase (ALT) was not significantly different (121.2 ± 39.3 vs. 128.1 ± 43.8 U/L). Mean serum hs-CRP in fatty liver patients was 3.1 ± 1.7 ng/mL, compared with 3.96 ± 1.6 ng/mL in NASH patients (*p* = 0.974).

Table 2. Characteristics of presumed NAFLD patients with elevated ALT ≥ 1.5 times based on performed the liver biopsy.

Variables	Liver biopsy		*p*-value
	Not done (N = 73)	Done (N = 30)	
Age (yrs)	52.5 ± 9.4	49.0 ± 8.5	0.074
Sex M/F (%)	36 (48.6)/38 (51.4)	20 (66.7)/10 (33.3)	0.080
BMI (kg/m^2)	27.6 ± 4.8	27.3 ± 4.2	1.000
Waist circumference (cm)	85.5 ± 8.0	88.0 ± 10.0	0.314
TG (mg/dL)	184.6 ± 80.6	197.9 ± 102.9	0.486
HDL (mg/dL)	42.4 ± 10.6	40.3 ± 8.90.345	
Ferritin (ng/mL)	334.0 ± 447.0	336.9 ± 258.4	0.975
hs-CRP (mg/L)	3.6 ± 6.4	3.6 ± 4.7	0.905
HOMA-IR	4.6 ± 4.9	5.0 ± 4.2	0.719
Insulin resistance (%)	57 (77.0)	28 (93.3)	0.172
History of diabetes (%)	23 (31.1)	6 (20.0)	0.010
History of hypertension (%)	37 (50.0)	9 (31.0)	0.122
History of dyslipidemia (%)	37 (50.0)	16 (55.2)	0.667
Metabolic syndrome (%)	31 (41.9)	12 (41.4)	1.000
ALT (U/L)	88.3 ± 3.6	128.3 ± 4.8	0.940

Table 3. Clinical variables in fatty liver patients and NASH patients.

Variables	Fatty Liver (n = 7)	NASH (n = 23)	*p*-value
Age (yrs)	49.3 ± 7.7	48.3 ± 8.9	0.700
BMI (kg/m^2)	26.3 ± 3.3	27.1 ± 3.6	0.449
Waist circumference (cm)	85.6 ± 5.25	89.7 ± 7.6	0.216
Ferritin (ng/mL)	169.2 ± 96	361.7 ± 263.2	0.107
HOMA-IR[#]	3.9 ± 1.4	4.0 ± 1.5	0.917
hs-CRP (mg/L)	3.1 ± 1.67	3.96 ± 1.6	0.974
ALT (U/L)	121.2 ± 39.3	128.1 ± 43.8	0.818
BMI (kg/m^2) ≥ 25	5 (71.4)	17 (73.3)	1.000
Waist circumference (cm) F ≥ 80 M ≥ 90	3 (42.9)	17 (83.9)	0.181
Metabolic syndrome	3 (42.9)	9 (39.1)	0.453
Insulin resistance (HOMA-IR ≥ 2)	6 (85.7)	22 (95.7)	0.410
History of diabetes (%)	3 (42.9)	3 (13.0)	0.120
History of hypertension (%)	2 (28.6)	6 (26.1)	1.000

Abbreviation: *NASH, nonalcoholic steatohepatitis.*
[#]*DM subjects not included.*

Table 4 displays characteristics of NAFLD patients with elevated ALT ≥1.5 times, based on severity of fibrosis. There were 20 mild fibrosis patients (F1-F2) (67.7%) and 10 advanced fibrosis patients (F3-F4) (33.3%). Mean age appeared to differ in mild fibrosis and advanced fibrosis (46.7 ± 8.2 and 53.4 ± 7.8, p = 0.047). Mean HOMA-IR was not higher in advanced fibrosis patients than in mild fibrosis patients (4.1 ± 1.1 vs. 3.6 ± 1.8, p = 0.175).

From table 5, NASH state 4 patients was not higher HOMA-IR than NASH state 3 patients (4.5 ± 1.6 vs. 2.9 ± 0.9, p = 0.086), which their serum ALT was significant lower (121.1 ± 35.7 vs. 141.3 ± 56.3, p = 0.047).

Table 4. Characteristics of NAFLD patients with liver biopsy, based on severity of fibrosis.

Variables	Mild fibrosis (F1-F2) (n = 20)	Advanced fibrosis (F3-F4) (n = 10)	*p*-value
Age (yrs)	46.7 ± 8.2	53.4 ± 7.8	0.047
BMI (kg/m^2)	26.8 ± 3.9	27.1 ± 2.6	0.127
Waist circumference (cm)	88.4 ± 10.0	89.4 ± 6.2	0.162
Ferritin (ng/mL)	301.8 ± 282.2	345.0 ± 150.8	0.375
HOMA-IR	3.6 ± 1.8	4.1 ± 1.1	0.175
hs-CRP (mg/L)	3.4 ± 4.4	2.6 ± 1.6	0.149
ALT (IU/mL)	127.1 ± 45.8	125.5 ± 36.3	0.371
BMI (kg/m^2) ≥ 25	14 (70.0)	8 (80.0)	0.682
Waist (cm) F ≥ 80 M ≥ 90	13 (65.0)	7 (70.0)	1.000
Metabolic Syndrome	7 (35.0)	5 (50.0)	0.461
Insulin resistance (HOMA-IR ≥ 2)	18 (90.0)	10 (100.0)	0.540
History of diabetes (%)	4 (20.0)	2 (20.0)	1.000

mean ± SD

Table 5. Characteristics of NASH patients based on Metteoni's classification.

Variables data	State 3 (n = 8)	State 4 (n = 15)	*p*-value
HOMA-IR	2.9 ± 0.9	4.5 ± 1.6	0.086
ALT (U/L)	141.3 ± 56.3	121.1 ± 35.7	0.047
Insulin resistance (HOMA-IR ≥ 2)	7 (87.5)	1 (100.0)	0.348

mean ± SD

DISCUSSION

In our present study, HOMA-IR in NASH patients was not higher than in fatty liver patients ($p = 0.917$). The study limitation may be due to small number of study patients. Most NAFLD and NASH patients were also overweight (BMI ≥25 kg/m^2).

One-hundred-and-three patients (64.8%) met criteria for liver biopsy, of whom only 30 (29.1%) gave informed consent for the procedure. The clinical and biochemical characteristics of patients with or without liver biopsy were not significantly different of the patients with liver biopsy, only six (20.7%) had DM which most patients (93.3%) had insulin resistance (HOMA-IR ≥2), in keeping with the general observation that NAFLD and NASH patients have insulin resistance.

In our recent study we found that serum ALT level in NAFLD and in NASH patients was not different. This parameter therefore, should not be used to differentiate these two groups of patients. Regarding inflammatory markers, such as serum ferritin and serum hs-CRP, these trend to be higher in NASH patients than in fatty liver patients, but not statistically significant.

In that recent study of ours we also found advanced fibrosis (F3-F4) in 33.3%, and NASH state 4, by Metteoni's classification in 50% of 30 patients who underwent liver biopsy. NASH state 4 was not higher HOMA-IR than NASH state 3 patients, but with a lower serum ALT. Thus, HOMA-IR could not used to predict the severity of NASH.

NAFLD patients trend to progress to more severe liver disease with its inherent complications. This trend could be more rapid in the presence of higher insulin resistance. The latter is a sensitive predictor of both progressive liver disease and severe extra-hepatic disease.

The association between insulin resistance and hepatic steatosis remains unclear[20]. In obese subjects, the primary abnormality may be genetically induced insulin resistance, with a secondary increase of serum triglyceride levels due to enhanced peripheral lipolysis. The resulting hepatic supply of fatty acids and insulin may increase triglyceride deposition in the liver[21]. In a recent study, insulin resistance as determined with HOMA model was associated with elevated serum alanine aminotransferase (ALT), irrespective of weight, body mass index and dietary intake[22]. Other

studies have shown that the higher the ALT level, the greater the risk of NASH[23,24]. There was some data indicating that patients with normal ALT may also have histological features at risk for disease progression[25,26].

Several studies have detailed the information on the relationship between histological and clinical findings in patients with NAFLD, demonstrating that the higher the number of components of the metabolic syndrome, the higher the risk of fibrosis and advanced disease[27].

In conclusion, liver biopsy remains the gold standard for diagnosing nonalcoholic steatohepatitis (NASH). HOMA-IR level cannot be used to separate NAFLD patients from NASH patients. A new and significant non-invasive tool to early detect NASH patients is to be awaited such as HOMA-IR or serum ferritin level. Although in our study there was a trend for higher level of HOMA-IR in more progressive NASH than in fatty liver patients, the difference was not statistically significant, possibly from the small sample size.

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