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Association of Insulin Resistance with Alanine Aminotransferase Activity in Patients with Nonalcoholic Fatty Liver Disease

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Abstract

Objective: Elevated liver enzymes have been recognized as a predictor for the development of metabolic syndrome and type 2 diabetes mellitus in patients with nonalcoholic fatty liver disease (NAFLD). However, the association of insulin resistance with alanine aminotransferase (ALT) activity remains largely unknown.

Patients and Methods: A total of 454 subjects attending the health examination center were enrolled from September 2005 through December 2006. Of them, 203 patients (male/female, 113/90; mean age, 50.9 years) were identified to have NAFLD. Their insulin resistance index was determined using the homeostasis model assessment (HOMA-IR). Elevated ALT level was defined as >30 IU/L for male and >19 IU/L for female subjects according to the new cut-off values.

Results: There were 113 subjects in the elevated ALT group and 90 in the normal ALT group. Age, gender and the prevalence of diabetes mellitus, hypertension and smoking history were comparable between these two groups. The elevated ALT group had higher readings for body mass index, triglyceride, total cholesterol, low-density lipoprotein cholesterol, insulin and HOMA-IR than the normal ALT group using univariate analyses. Multivariate logistic regression analyses showed that only log(HOMA-IR) was positively associated with elevated ALT level in the NAFLD patients (adjusted odds ratio, 5.04; 95% confidence interval, 1.56–16.27).

Conclusion: Our data showed that there was an association between insulin resistance and serum ALT levels in NAFLD patients, independent of other metabolic factors. However, further longitudinal studies are needed to clarify the causal relationship between insulin resistance and increased ALT activity in patients with NAFLD. (*Tzu Chi Med J* 2008;20(4):275–279)

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1. Introduction

Nonalcoholic fatty liver disease (NAFLD), already the most common liver disease in the United States, can be expected to increase parallel with the epidemics of obesity and type 2 diabetes mellitus [1]. The term refers to a spectrum of disorders from simple steatosis to more severe manifestations including steatohepatitis, fibrosis and cirrhosis [2]. In addition, subjects with NAFLD have been reported to have high prevalence of metabolic syndromes and high risk of cardiovascular diseases [3–6]. Therefore, NAFLD is emerging as a global health problem.

The pathogenesis of NAFLD has been proposed to result from two physiologic events [7]. The first event is thought to be insulin resistance, leading to the accumulation of triglycerides in hepatocytes and subsequent lipid peroxidation. The second event includes increased oxidative stress, cytokine release and, ultimately, Fas ligand-mediated liver injury. Serum alanine aminotransferase (ALT) level is recognized as a clinical marker of liver injury and may reflect the outcome of the second event in the pathogenesis of NAFLD [8]. Furthermore, increased ALT activity has been recognized as a predictor of the development of diabetes and metabolic syndromes in NAFLD patients [9,10]. However, the association of increased ALT activity with insulin resistance or metabolic factors in NAFLD patients remains unclear. In this cross-sectional study, we explored demographic and metabolic factors associated with increased ALT activity in NAFLD patients.

2. Materials and methods

2.1. Subjects and methods

A total of 454 subjects attending the health examination center at the Buddhist Tzu Chi General Hospital in Taipei, Taiwan with informed consent were enrolled from September 2005 through December 2006. Of them, 203 subjects who fulfilled the diagnostic criteria of NAFLD were included in the study. The diagnostic criteria of NAFLD included no excessive alcohol intake (<140 g/week), no chronic viral hepatitis (negative for hepatitis B surface antigen and antibody against hepatitis C virus), no known etiologies of liver diseases, and the presence of fatty liver on liver ultrasound examinations [11]. Age, gender, body weight, body height, body mass index (BMI), and waist circumference were recorded. Biochemical tests including fasting plasma glucose, ALT, aspartate aminotransferase (AST), triglyceride, total cholesterol, high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were determined using standard laboratory procedures. Serum insulin levels were assessed using an enzyme-linked immunoassay (Bayer Corp., Tarrytown, NY, USA). Insulin resistance (IR) was calculated using the homeostasis model assessment for insulin resistance (HOMA-IR) index. HOMA-IR = fasting insulin (mU/L) × fasting glucose (mg/dL) × 0.05551/22.5 [12]. Those with diabetes mellitus were defined as having previous diabetic history or fasting plasma glucose level >126 mg/dL. Elevated ALT levels were defined as >30 IU/L for male subjects and >19 IU/L for female subjects according to the new cut-off values [13].

2.2. Ultrasound examination

A GE LOGIO 5 Pro Ultrasound system (GE Medical Systems, Seoul, Korea) with a 4-MHz electronic probe was used to examine the livers. The presence of fatty liver was assessed on the basis of ultrasonographic findings of a bright liver, increased echogenicity with evident contrast between the liver and kidney, vascular blurring and deep attenuation of the ultrasound signal as previously described [14,15].

2.3. Ethical considerations

The study was conducted in accordance with the principles of the 1975 Declaration of Helsinki and approved by the Ethics Committee of the Buddhist Tzu Chi General Hospital in Taipei, Taiwan. Serum samples were obtained after receiving informed consent from each subject.

2.4. Statistical analysis

Data were expressed as mean ± standard deviation. HOMA-IR distributed as a screw pattern, thus was log-transformed for subsequent analysis. χ^2 and t tests were used to analyze categorical and continuous variables, respectively. Statistical analyses were performed using Stata version 8.0 (Stata Corp., College Station, TX, USA) and all tests were two-sided. A value of $p<0.05$ was considered statistically significant.

3. Results

3.1. Study population and demographic data

A total of 203 NAFLD patients (men/women, 113/90; mean age, 50.9 years) were included for final analysis. The prevalence of metabolic syndromes according to the modified Adult Treatment Panel III diagnostic criteria was 32% in these NAFLD patients. There were 113 subjects in the elevated ALT group and 90 in the normal ALT group. The demographic and metabolic

Table 1 — Comparisons of demographic and metabolic features between nonalcoholic fatty liver disease patients with and without ALT elevation*

Features	Elevated ALT group (N=113)	Normal ALT group† (N=90)	p
Age (yr)	50.8±1.0	51.0±1.4	0.89
Male	57 (50)	56 (62)	0.09
Body mass index (kg/m^2)	26.0±0.3	24.8±0.4	0.02
Waist circumference (cm)	85.3±0.9	84.4±1.2	0.54
Fasting blood glucose (mg/dL)	105.9±3.1	100.0±3.3	0.20
Insulin (mU/L)	10.7±0.7	8.0±0.6	<0.01
HOMA-IR	2.8±0.2	2.1±0.2	0.02
AST (IU/L)	29.7±1.5	20.2±1.2	<0.01
ALT (IU/L)	42.3±2.2	19.6±0.6	<0.01
Triglyceride (mg/dL)	168.6±10.7	128.4±8.0	<0.01
Total cholesterol (mg/dL)	203.1±4.0	186.1±4.0	<0.01
LDL-C (mg/dL)	140.1±3.8	127±4.0	0.01
HDL-C (mg/dL)	50.6±1.2	51.8±1.7	0.56
Diabetes mellitus	17 (15.04)	9 (10.00)	0.29
Hypertension	17 (15.18)	18 (20.00)	0.37
Smoking habit	16 (14.16)	15 (16.67)	0.62
Metabolic syndrome	44 (38.94)	21 (23.33)	0.02

*Data presented as mean±standard deviation or n (%);†normal ALT level is <30 U/L in males and <19 U/L in females. ALT = alanine aminotransferase; HOMA-IR = homeostasis model assessment-insulin resistance; AST = aspartate aminotransferase; LDL-C = low-density lipoprotein cholesterol; HDL-C = high-density lipoprotein cholesterol.

features of the two groups are shown in Table 1. These two groups of patients were comparable in terms of age, gender and the prevalence of diabetes mellitus, which are the major confounding factors of insulin resistance. In addition, the prevalence of hypertension and smoking history did not differ between the patients with and without ALT elevation.

3.2. *Factors associated with elevated serum ALT levels*

By univariate analyses, the prevalence of metabolic syndromes, serum insulin levels and HOMA-IR were significantly higher in the elevated ALT group than in the normal ALT group. Regarding the lipid profile, subjects with elevated ALT levels had increased serum triglyceride, total cholesterol and LDL-C levels compared to those with normal ALT levels. In addition, NAFLD patients with elevated ALT levels had higher BMI than those with normal ALT levels. In contrast, fasting plasma glucose levels, serum HDL-C levels and waist circumference were comparable between the two groups of patients. Using multiple logistic regression analyses, the factors associated with ALT elevation in the NAFLD patients are shown in Table 2. The model was adjusted for BMI, triglyceride, total cholesterol, LDL-C and log(HOMA-IR). In addition, the association holds true even after excluding patients with diabetes mellitus for analyses. Our data showed that only log(HOMA-IR) was positively associated with elevated ALT levels in patients with NAFLD (adjusted odds ratio, 5.04; 95% confidence interval, 1.56–16.27).

Table 2 — Factors associated with ALT elevation in nonalcoholic fatty liver disease patients by multiple logistic regression analyses*†

	Adjusted OR (95%CI)
Body mass index (kg/m^2)	1.02 (0.93–1.12)
Total cholesterol (mg/dL)	1.01 (0.99–1.04)
Triglyceride (mg/dL)	1.00 (1.00–1.01)
Log(HOMA-IR)	5.04 (1.56–16.27)‡
LDL-C (mg/dL)	1.00 (0.97–1.02)

*Model is adjusted for body mass index, total cholesterol, triglyceride, log(HOMA-IR) and LDL-C; †normal ALT level is <30 U/L in males and <19 U/L in females; ‡p<0.05. ALT = alanine aminotransferase; HOMA-IR = homeostasis model assessment-insulin resistance; LDL-C = low-density lipoprotein cholesterol.

4. Discussion

Little is known about the association of insulin resistance with serum ALT levels except in special groups such as obese subjects and patients with HIV lipodystrophy (16–19). Researchers in a previous study showed that elevated ALT levels and fatty livers were independently associated with increased risk of metabolic syndromes (20). Therefore, it has been speculated that insulin resistance may have dual effects on inducing fat accumulation in liver cells and initiating the inflammatory cascade by which liver injury is caused. However, the authors failed to provide direct evidence to prove the positive association between insulin resistance and elevated serum ALT levels. In our study, we found that elevated ALT levels were significantly associated with insulin resistance and the components of metabolic syndrome in NAFLD patients using univariate

analysis. In addition, multivariate logistic regression analysis showed that insulin resistance was the only factor that positively correlated with increased ALT activity after adjustment of several demographic and metabolic variables. It is generally believed that insulin resistance plays a central role in the pathogenesis of NAFLD. Our data further suggested that insulin resistance may be directly associated with the inflammatory process of the liver in NAFLD patients. In addition, a recent case-control study indicated that pioglitazone, an insulin sensitizer, normalized liver aminotransferase and improved histological findings especially in the grade of necroinflammation in subjects with nonalcoholic steatohepatitis (NASH) (21). Taking these lines of evidence together, insulin resistance also plays an important role in the initiation of the inflammatory cascade (second event) to incite the aggressive form of NAFLD called NASH (22).

Although several studies have indicated the impact of elevated serum ALT levels on the risk of the subsequent occurrence of diabetes mellitus, metabolic syndromes and coronary heart diseases (23–25), the mechanisms to explain these associations remain controversial. It is known that insulin resistance plays a key role in the development of diabetes mellitus and metabolic syndromes. However, whether insulin resistance could explain the relationship between increased ALT activity and diabetes mellitus as well as metabolic syndromes awaits further examinations. A community-based study was conducted in Taiwan to determine the prevalence and risk factors of NAFLD in the general population (26). The results showed that NAFLD patients with elevated ALT levels did not differ from those with normal ALT levels in the prevalence of each metabolic derangement. Thus, serum ALT level might not be a good predictor of metabolic abnormalities in NAFLD patients. On the contrary, another group of researchers investigated the relative contribution of metabolic syndromes and insulin resistance to increased ALT activity in NAFLD patients, and defined the number of metabolic syndromes as central obesity, plus 0 to 4, according to the number of other four metabolic syndrome components by the new International Diabetes Federation (IDF) definition (27). They found that the number of metabolic syndrome components, BMI, hs-CRP and insulin resistance were predictors of increased ALT activity in patients with NAFLD (28). In this study, our data further proved the association of insulin resistance with increased ALT levels in NAFLD patients, independent of other metabolic factors which could confound the value of insulin resistance, suggesting that increased ALT levels might serve as a surrogate marker of insulin resistance and reflect the increased risk of diabetes mellitus, metabolic syndromes or even cardiovascular diseases. Researchers in two recent studies also found an early association of ALT with insulin resistance in women (29,30). Thus, NAFLD patients with increased ALT activity deserve aggressive treatment of coexisting metabolic disorders and strict lifestyle modification to improve insulin sensitivity and reduce the risk of cardiovascular diseases.

Age, gender, and the presence of diabetes mellitus are the major confounding factors of insulin resistance (31). We enrolled consecutive subjects from a health examination center with informed consent to avoid selection bias, and our results showed that the aforementioned factors were comparable between the NAFLD patients with and without ALT elevation. Accordingly, we objectively demonstrated the correlation between insulin resistance and increased ALT activity in NAFLD patients. However, a number of potential limitations should be considered. For example, the case number in this cohort was relatively small, and the cross-sectional study design did not show the causal relationship. Therefore, further large-scale and longitudinal studies are needed to clarify the causal relationship between insulin resistance and increased ALT activity in patients with NAFLD.

In summary, our findings suggested that insulin resistance is associated with increased ALT activity in patients with NAFLD, and is independent of other metabolic factors. These observations will lay the groundwork for further studies to clarify the association of insulin resistance with liver inflammation in the pathogenesis of NAFLD. Nevertheless, the link between insulin resistance and liver damage needs prospective and mechanistic studies.

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