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Relationship between Hemoglobin Levels and Risk for Suspected Non-Alcoholic Fatty Liver in Taiwanese Adults

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Abstract

Body iron levels have recently been shown to be a strong predictor for non-alcoholic fatty liver disease (NAFLD). The aims of this study were to investigate the prevalence of NAFLD in a general adult population, and to investigate the relationship between body iron levels, NAFLD and the metabolic syndrome (MetS). 2186 adults participated in the third National Nutrition and Health Survey in Taiwan (NAHSIT, 2005-2008). The participants underwent anthropometry measurements and phlebotomy after an overnight fast, and those with excessive alcohol intake, iron overload of serum ferritin > 600 ng/ml, hepatitis viral infection and hepatocellular carcinoma were excluded. Suspected NAFLD was diagnosed by three alanine transaminase (ALT) cut-points: cut-point 1: serum ALT > 40 U/l; cut-point 2: ALT ≧ 25 U/l for male and ALT ≧ 17 U/l for female; and cut-point 3: ALT ≧ 35 U/l for male and ALT ≧ 26 U/l for female. The prevalence proportion of suspected NAFLD among Taiwanese adults was 6.6% (cut-point 1), 36% (cut-point 2); and 14.3% (cut-point 3). Body iron levels were significantly higher in individuals with suspected NAFLD compared with those without. Distribution of hemoglobin levels, but not serum ferritin levels, by decade of age showed strong correlation with the prevalence of suspected NAFLD in individuals with MetS. Multivariate adjusted odds ratio (OR) showed that the best predictors for suspected NAFLD with the MetS were hemoglobin [OR 1.43 (1.21-1.68); $P < 0.0001$] and hyperlipidemia [OR 1.52 (1.19-1.94); $P = 0.0007$]. In individuals without MetS, the adjusted OR of suspected NAFLD was markedly higher for hemoglobin [OR 1.25 (1.12-1.41); $P < 0.0001$]. In conclusion, adults with high hemoglobin levels (14.4 μg/dl for male and 13.2 μg/dl for female) are at the greatest risk for developing abnormal liver function. Hemoglobin test should be considered as a part of clinical evaluation for patients with NAFLD.

Key Words: alanine transaminase, body iron levels, metabolic syndrome, non-alcoholic fatty liver disease

Introduction

Nonalcoholic liver disease (NAFLD) has become increasingly recognized as a public health problem in Asia including Taiwan (1, 22). The rising incidence of NAFLD coincides with a marked increase in obesity

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(38). NAFLD is defined as fat accumulation in the liver exceeding 5% to 10% by weight in the absence of alcohol abuse. Symptom ranges from simple steatosis to nonalcoholic steatohepatitis (NASH), and to advanced fibrosis and cirrhosis. The etiology of NAFLD remains unknown. However, there is a strong association with age, gender, body mass index (BMI), body iron status and individual components of the metabolic syndrome (MetS) such as type 2 diabetes, hypertension and hyperlipidemia (1, 7, 22, 48). For this reason, NAFLD has been suggested as a hepatic manifestation of MetS (35, 50).

The prevalence of NAFLD in adults is not clear but existing studies have suggested that NAFLD affects 11-52% of the adult population in Asia (3, 10, 31). However, the prevalence rates are higher in subgroups such as obese individuals, diabetic patients and males. Lin *et al*. studied records of 4,360 adults who completed annual health checkup and reported that 52% had ultrasound evidence of steatosis (31). This rate is similar to a study of 1,016 Taiwanese police officers which reported 52.2% ultrasound-proven steatosis (45). Obesity and type 2 diabetes are the two most important risk factors for the development of NAFLD (10, 31). The prevalence of NAFLD in obese individuals ranges from 63% in obese adolescents (18) to 80% in obese adults (21). The prevalence of NAFLD in patients with type 2 diabetes is estimated to be >75% (26). Insulin resistance is thought to promote excessive hepatic fat accumulation leading to the development of NAFLD. Although prevalence of NAFLD is closely related to BMI, a high percentage (7.2%-15%) of NAFLD subjects has been found in non-obese Chinese adults (10, 32, 48).

Because there is no suitable diagnostic tool for the epidemiology of NAFLD, serum alanine aminotransferases (ALT) activity has been used as a surrogate marker for histological diagnosis of NAFLD in population-based studies (8, 10, 40). NAFLD is the most common cause of asymptomatic elevation of ALT levels (4). ALT is a cytosolic enzyme found predominantly in hepatocytes. When damage occurs, ALT is released from liver cells into the bloodstream, making it a more specific marker of steatohepatitis (4). Therefore, ALT values above the upper normal limit of >40 U/l typically reflect hepatic damage, and is a more specific marker of NASH and cirrhosis (4). Recently, the healthy thresholds for serum ALT levels in predicting NAFLD has been challenged (9, 24, 28, 37, 42). It has been noticed that liver enzyme levels in NAFLD patients fluctuate and ALT levels maybe within the reference interval in up to 78% of patients with diagnosed NAFLD (1, 8). As has been reported previously, individuals with hepatic steatosis may have normal serum ALT levels (47). An attempt to appropriately define ALT threshold value for diagnosis of NAFLD showed that the healthy threshold for serum ALT levels is much lower than the currently accepted cut-point of ALT > 40 U/l (28, 37, 42). In addition, the appropriate ALT threshold may also be influenced by gender, age as for children *vs*. adults, BMI and metabolic status.

NAFLD is considered to be the hepatic manifestation of MetS. However, not all patients with NAFLD develop MetS and the risk factors for NAFLD may differ in individuals with or without diagnosed MetS (20, 29, 50). Body iron levels play a critical role in NAFLD and MetS (6, 16). Yilmaz and colleagues studied the risk factors of 357 biopsy-proven NAFLD and showed hemoglobin as the only predictor closely associated with NASH and fibrosis for the NAFLD patients without MetS (50). By contrast, insulin resistance and diabetes were independently associated with NASH in NAFLD patients diagnosed with MetS. Serum hemoglobin α and β subunits have been identified as biomarkers for biopsy-proven NAFLD in adults (44, 52). Yu *et al*. followed a total of 6,944 initially NAFLD-free Chinese subjects for 3 years and found hemoglobin to be a strong predictor for NAFLD (52). Furthermore, increased serum ferritin levels have been reported as an independent predictor of liver damage (severe hepatic fibrosis/NASH) in patients with biopsy-proven NAFLD (25, 46). A 5-year follow up study on 5,562 lean Chinese adults who were initially free of NAFLD found that hemoglobin and platelet counts were significantly associated with the development of NAFLD (48). Collectively, these evidences suggest that body iron levels are important risk factor for NAFLD and incorporation of body iron levels as a predictor may help to prevent liver damages in patients with NAFLD (46).

The present study explored the relationships between suspected NAFLD, defined by elevated ALT, body iron levels and MetS in adults recruited in the Nutrition and Health Survey in Taiwan (NAHSIT) 2005-2008. The objectives of the study were: [1] to compare the prevalence of suspected NAFLD among Taiwanese adults using various cut-points for elevated ALT, and [2] to investigate the risk factors associated with suspected NAFLD in relation to metabolic status.

Materials and Methods

Study Design

The Third National Nutrition and Health Survey in Taiwan (NAHSIT 2005-2008, adults) was funded by the Department of Health to provide continued assessment of health and nutritional status of residents in Taiwan. The nationwide survey was conducted using a multi-staged, stratified and clustered sampling scheme which included a wide range of age

groups across the whole of Taiwan. The present study analyzed data on adults, aged ≧19 years old. This study was approved by the Research Ethics Committee of Taipei Medical University (20120303) and Academia Sinica (AS-IRB01-07020). Written informed consents were obtained from all participants.

Sample Inclusion and Exclusion

Exclusion criteria were as follows: [1] individuals with missing data; [2] individuals with abnormal serum ferritin >600 ng/ml, which was used as a surrogate marker for chronic inflammation; [3] disease history of hepatitis viral C and B infection, hepatocarcinoma, nephritis and cancer; and [4] excessive alcohol intake, defined by alcohol intake <20 g/week for women or <30 g/week for men. As such, a total of 2,186 adult participants (971 male and 1215 female) were selected for analysis.

Laboratory Measurements

Biochemistry data were obtained from 8-hour fasting blood samples. Heparinized whole blood was collected for on-site measurement of hemoglobin. Peripheral venous blood samples were collected in tubes containing EDTA, centrifuged at 4°C and stored serum at -80°C until analysis. Clinical biochemistry included: serum cholesterol (total cholesterol, LDL-C and HDL-C), triglycerides (TG), fasting blood glucose, uric acids (UA), C-reactive protein (CRP), creatinine, homocysteine, ALT, aspartate transaminase (AST), amylase, blood urea nitrogen (BUN) , alkaline phosphatase (ALKP) and iron parameters, *i.e.* hemoglobin, serum iron, serum ferritin, total iron binding capacity (TIBC).

Definitions

Suspected NAFLD was defined by elevated ALT values based on various approaches: cut-point 1: serum ALT > 40 U/l (9); cut-point 2: ALT ≧ 25 U/l for male and ALT ≧ 17 U/l for female (37); and cut-point 3: ALT ≧ 35 U/l for male and ALT ≧ 26 U/l for female (28). BMI was calculated as mass (kg)/[height (m)]^2. Hypertension was defined as systolic blood pressure (SBP) ≧ 140 or diastolic BP (DBP) ≧ 90 mmHg, or the use of antihypertensive medications. Type 2 diabetes was defined as fasting plasma glucose (FPG) ≧126 mg/dl, or the use of antidiabetic drugs. Hyperlipidemia was classified by the presence of any criteria listed below: [1] total cholesterol ≧ 240 mg/dl; [2] TG ≧ 200 mg/dl; [3] LDL ≧ 160 mg/dl; [4] HDL <40 mg/dl, or [5] current use of antihyperlipidemia drugs. To estimate the association between ALT elevation and metabolic risk, MetS was defined based on the modified National Cholesterol Education Program Adult Treatment Panel III Criteria for Asia Pacific*. Individuals with the presence of ≧3 criteria listed below were classified as having MetS: [1] overweight as BMI ≥ 24 kg/m^2 and obese as BMI ≥ 27 kg/m^2; [2] waist circumference ≥90 cm in men and ≥80 cm in women; [3] TG ≧ 150 mg/dl [4] HDL <40 mg/dl for men and <50 mg/dl for women; [5] SBP ≧ 130 mmHg, or DBP ≧ 85 mmHg, or current use of antihypertensive drugs; and [6] FPG ≧ 100 mg/dl, or current use of antihyperglycemic drugs.

Statistical Analyses

Statistical analyses were performed using the statistical software package SAS version 9.22. Categorical data were presented as number and percentage. Continuous data were presented as mean and standard deviation. Differences between two independent samples were analyzed by the Wilcoxon rank-sum test for the nonparametric data. For Table 2-4, suspected NAFLD was diagnosed using NAFLD cut-point 2 as defined above (37). Logistic regression models were used to assess the independent effects of known risk factors, including anthropometry, iron parameters, components of MetS and inflammatory markers, on the odds of suspected NAFLD prevalence. The predicting values of hemoglobin and serum ferritin levels for suspected NAFLD were evaluated by plotting the area under the receiver operating characteristic (ROC) curve (AUC) and calculating the sensitivity and specificity. P values < 0.05 were considered statistically significant.

Results

The mean age of participants in this study was 53.7 ± 18.3 years: male 54.7 ± 18.7 and female 53.0 ± 17.9. Mean BMI was 24.4 ± 3.9 kg/m^2: male 24.5 ± 3.5 and female 24.3 ± 4.2. Mean ALT was 21.1 ± 17.5 U/l: male 23.7 ± 18.6 and female 19.0 ± 16.3. Mean Hb was 13.5 ± 1.6 μg/dl: male 14.5 ± 1.4 and female 12.7 ± 1.3, and mean serum ferritin were 134.6 ± 105.8 mg/dl: male 176.8 ± 108.9 and female 100.2 ± 89.6. The prevalence of MetS, hyperlipidemia, hypertension and type 2 diabetes was: 31.7%, 28.6%, 23.5% and 8.7%, respectively. The prevalence of suspected NAFLD defined by three approaches was: [1] 6.6%

*Expert Panel on Detection E and Treatment of High Blood Cholesterol in A. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *J.A.M.A.* 285: 2486-2497, 2001.

Table 1. Clinical characteristics of the Taiwanese subjects with suspected NAFLD defined by elevated serum ALT concentrations (n = 2186)

	NAFLD 1[#]	NAFLD 2[§]	NAFLD 3[※]
Suspected NAFLD (%)	144 (6.6%)	787 (36.0%)	313 (14.3%)
Male sex (%)	91 (63%)	496 (63%)	134 (42.8%)
Age (yrs)	48.2 (17.6)	54.2 (16.4)	52.6 (17.1)
BMI (kg/m^2)	27.5 (4.2)	25.9 (4.0)	26.8 (4.3)
ALT (U/l)	67.3 (37.8)	33.1 (23.6)	48.7 (31.0)
Hemoglobin (μg/dl)	14.4 (1.4)	13.8 (1.5)	13.9 (1.4)
Serum Ferritin (ng/ml)	210.7 (124.0)	159.4 (113.4)	176.6 (118.7)

[#]Criteria 1: suspected NAFLD defined by ALT > 40 U/l; [§]Criteria 2: suspected NAFLD by gender: ALT ≧ 25 U/l in male; ALT ≧ 17 U/l in female; [※]Criteria 3: Suspected NAFLD by gender: ALT ≧ 35 U/l in male; ALT ≧ 26 U/l in female; [†]Categorical data are presented as number (%); continuous data are presented as mean (standard deviation).

(63% for male) for cut-point 1; [2] 36% (63% for male) for cut-point 2; and [3] 14.3% (43% for male) for cut-point 3 (Table 1). We next examined the prevalence proportion of suspected NAFLD by decade of age. Using cut-point 2 and cut-point 3, the highest rate was found in subjects aged 50-59 and 60-69 years; 45.8% and 44.3% for criteria 2 and 17.7% and 15.9% for criteria 3; respectively (Fig. 1A). When using cut-point 1, the highest rate was found in subjects aged < 40 years (9.3%) (Fig. 1A).

We next investigated the association between suspected NAFLD and body iron levels in relation to MetS. In individuals with MetS, the highest rate of suspected NAFLD was found in subjects aged <40 years; 32.8% using cut-point 1, 73.7% using cut-point 2 and 45.9% using cut-point 3 (Fig. 1B). Distribution of hemoglobin levels by decade of age showed strong correlation with the prevalence of suspected NAFLD (Fig. 1B); in contrast, serum ferritin levels did not (Fig. 1D). In individuals without MetS, the highest prevalence rate of suspected NAFLD was found in subjects aged 60-70 years using gender-specific ALT cut-point 2 and 3; 40.3% and 12.4%, respectively (Fig. 1C). The distribution of serum ferritin and hemoglobin levels by decade of age was associated with the prevalence proportion of suspected NAFLD defined by cut-point 2 (Fig. 1, C and E).

We next defined suspected NAFLD using ALT cut-point 2. Adults with suspected NAFLD had higher waist circumference, BMI, individual components of MetS (SBP, DBP, total cholesterol, TG, LDL-cholesterol and fasting plasma glucose), iron status (serum ferritin, TIBC and hemoglobin), UA, creatinine, homocysteine, ALKP, lower HDL-cholesterol and higher male sex (all $P < 0.001$; data not shown). The results of univariate analysis showed that most of the analyzed variables, including BMI, serum ferritin, hemoglobin, individual components of MetS, hypertension, hyperlipidemia, UA, CREA, homocysteine and ALKP, were associated with the risk of suspected NAFLD with the exception of amylase, BUN and CRP (all $P < 0.05$; Table 2). In multivariate analysis, the adjusted OR was substantially higher for hyperlipidemia [OR 1.52 (1.19-1.94); $P = 0.0007$], Hb [OR 1.32 (1.20-1.45); $P < 0.0001$], BMI [OR 1.12 (1.08-1.15); $P < 0.0001$], UA [OR 1.12 (1.03-1.21); $P = 0.0048$], serum ferritin [OR 1.004 (1.003-1.006); $P < 0.0001$], and ALKP [OR 1.014 (1.009-1.019); $P < 0.0001$] (Table 3; Multivariate model; all subjects). We next separated individuals according to the presence of MetS. In individuals without MetS, the adjusted OR was markedly higher for hemoglobin [OR 1.25 (1.12-1.41); $P < 0.0001$], BMI [OR 1.14 (1.09-1.19); $P < 0.0001$], UA [OR 1.16 (1.04-1.29); $P = 0.0049$], ALKP [OR 1.016 (1.009-1.023); $P < 0.0001$] and serum ferritin [OR 1.004 (1.003-1.006); $P < 0.0001$] [(multivariate model; MetS (-)]. In individuals with MetS, the best predictor for suspected NAFLD were hemoglobin [OR 1.43 (1.21-1.68); $P < 0.0001$] and hyperlipidemia [OR 1.52 (1.19-1.94); $P = 0.0007$], respectively [(multivariate model; MetS (+)]. By the ROC analysis, a cut-off value of hemoglobin ≥14.4 μg/dl for male and ≥13.2 μg/dl for female gave a sensitivity and specificity of 74% *vs*. 78.2% and 48.4% *vs*. 49.3%; respectively (Table 4; all subjects). The AUC values for hemoglobin to predict suspected NAFLD were 0.645 for male and 0.643 for female. The sensitivity and the ROC values were higher for patients with MetS compared with those without (Table 4).

Discussion

In the present study, we demonstrated that the prevalence proportion of suspected NAFLD affected 6.6%-36% Taiwanese adults depending on the ALT cut-points. Individuals with suspected NAFLD had higher body iron levels and were characterized by the presence of multiple metabolic disorders such as

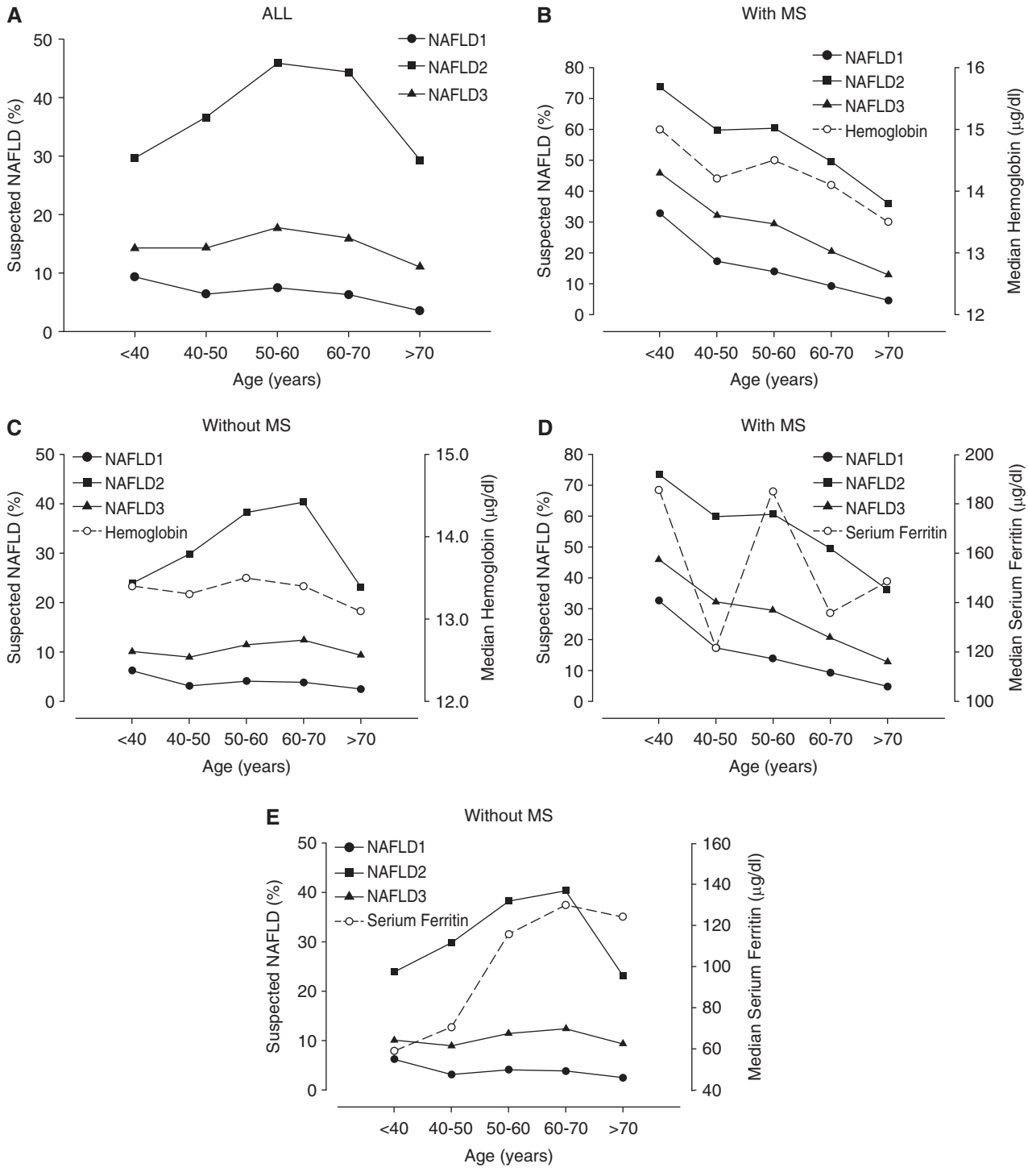

Fig. 1. Association between body iron status and unadjusted prevalence of suspected NAFLD by decade of age for Taiwanese adults aged ≧19 years old (n = 2186), defined using three cut-points for elevated ALT. (A) Trends in the prevalence of suspected NAFLD. (B-E) Association between the prevalence of suspected NAFLD and hemoglobin levels in the presence (B) or absence of MetS (C) (n = 687 *vs*. n = 1478), and serum ferritin levels in the presence (D) or absence of the MetS (E) (n = 687 *vs*. n = 1478).

dyslipidaemia and central obesity. Distribution of hemoglobin levels by decade of age showed strong correlation with the prevalence of suspected NAFLD using gender-specific cut-points. Multivariate analysis showed that hemoglobin level is a good predictor for individuals with suspected NAFLD with or without the MetS.

The liver is an important organ for iron and lipid

Table 2. Age- and gender-adjusted univariate analysis of odds ratio (OR) and 95% confidence interval (95% CI) for risk factors associated with suspected NAFLD

	All				MetS (-)				MetS (+)			
	*OR	95% CI		*P*-value	OR	95% CI		*P*-value	OR	95% CI		*P*-value
BMI (kg/m^2)	1.192	1.161	1.225	< 0.0001	1.194	1.152	1.238	< 0.0001	1.063	1.011	1.118	0.0163
Waist (cm)	1.058	1.048	1.069	< 0.0001	1.052	1.038	1.066	< 0.0001	1.021	1.002	1.041	0.0282
Serum TIBC (μg/dl)	1.004	1.002	1.006	0.0002	1.003	1.000	1.005	0.0304	1.004	1.000	1.008	0.0331
Serum ferritin (ng/ml)	1.005	1.004	1.006	< 0.0001	1.005	1.004	1.007	< 0.0001	1.004	1.002	1.005	< 0.0001
Transferrin saturation (%)	1.012	1.004	1.019	0.0018	1.016	1.007	1.025	0.0003	1.013	0.998	1.029	0.0883
Hemoglobin (ug/dl)	1.538	1.417	1.670	< 0.0001	1.429	1.288	1.584	< 0.0001	1.467	1.275	1.689	< 0.0001
Sytolic BP (mmHg)	1.017	1.011	1.022	< 0.0001	1.012	1.005	1.020	0.0014	1.005	0.996	1.014	0.2974
Diastolic BP (mmHg)	1.031	1.022	1.039	< 0.0001	1.02	1.009	1.032	0.0004	1.014	1.000	1.029	0.0578
Hypertension[&]	1.563	1.259	1.941	< 0.0001	1.013	0.730	1.406	0.9374	1.275	0.893	1.820	0.1805
Total cholesterol (mg/dl)	1.009	1.006	1.011	< 0.0001	1.008	1.005	1.012	< 0.0001	1.007	1.003	1.011	0.0014
LDL- cholesterol (mg/dl)	1.006	1.004	1.009	< 0.0001	1.008	1.004	1.011	< 0.0001	1.002	0.998	1.007	0.2484
Triglyceride (mg/dl)	1.007	1.006	1.009	< 0.0001	1.009	1.006	1.012	< 0.0001	1.003	1.001	1.005	0.0014
Hyperlipidemia[◎]	2.726	2.235	3.325	< 0.0001	2.220	1.677	2.939	< 0.0001	1.752	1.267	2.423	0.0007
HDL cholesterol (mg/dl)	0.983	0.977	0.990	< 0.0001	0.994	0.985	1.002	0.1587	1.008	0.994	1.023	0.2499
Fasting glucose (mg/dl)	1.008	1.005	1.011	< 0.0001	1.005	0.999	1.010	0.0807	1.194	0.843	1.692	0.3173
Type 2 diabetes[※]	1.599	1.245	2.055	0.0002	1.151	0.736	1.802	0.5371	1.004	1.000	1.008	0.0282
Amylase (mg/dl)	0.998	0.995	1.002	0.2911	1.002	0.998	1.006	0.2822	1.000	0.994	1.007	0.9778
BUN (mg/dl)	0.995	0.974	1.015	0.5984	1.014	0.986	1.044	0.3306	0.982	0.952	1.014	0.2666
UA (mg/dl)	1.288	1.210	1.370	< 0.0001	1.323	1.212	1.444	< 0.0001	1.086	0.985	1.198	0.0988
CRP (ng/ml)	1.051	0.872	1.267	0.6014	0.971	0.772	1.221	0.7999	0.987	0.665	1.466	0.9495
CREA (mg/dl)	0.494	0.313	0.778	0.0023	0.559	0.290	1.079	0.0829	0.523	0.262	1.044	0.0662
Homocysteine (umol/dl)	0.958	0.940	0.977	< 0.0001	0.945	0.919	0.972	< 0.0001	0.967	0.937	0.997	0.0307
ALKP (mg/dl)	1.019	1.014	1.023	< 0.0001	1.02	1.014	1.026	< 0.0001	1.008	1.001	1.015	0.0185

[&]Hypertension was defined as SBP ≧ 140 or DBP ≧ 90 mmHg or the use of antihypertensive medications; [◎]Hyperlipidemia: presence of any criteria: (1) total cholesterol ≧240 mg/dl; (2) TG ≧ 200 mg/dl; (3) LDL ≧ 160 mg/dl; (4) HDL <40 mg/dl or (5) the use of antihyperlipidemia drugs; [※]Diabetes: Fasting serum glucose ≧126 mg/dl or the use of antdiabetic drugs.

Table 3. Age- and gender-adjusted odds ratio and 95% confidence intervals (CI) for suspected NAFLD with or without the metabolic syndrome (MetS)

	All				MetS (-)				MetS (+)			
	OR	95% CI		*P*-value	OR	95% CI		*P*-value	OR	95% CI		*P*-value
BMI(kg/m^2)	1.121	1.085	1.158	< 0.0001	1.142	1.097	1.190	< 0.0001	1.036	0.976	1.099	0.2414
Serum TIBC(μg/dl)	1.006	1.003	1.008	< 0.0001	1.005	1.002	1.008	0.003	1.008	1.003	1.013	0.0024
Serum Ferritin (ng/ml)	1.004	1.003	1.006	< 0.0001	1.004	1.003	1.006	< 0.0001	1.004	1.002	1.006	< 0.0001
Hemoglobin (ug/dl)	1.322	1.204	1.452	< 0.0001	1.259	1.122	1.412	< 0.0001	1.430	1.214	1.686	< 0.0001
Hypertension	1.027	0.783	1.346	0.8495	0.792	0.529	1.186	0.2577				
Hyperlipidemia	1.525	1.194	1.948	0.0007	1.330	0.953	1.856	0.0931	1.586	1.091	2.305	0.0156
HDL cholesterol (mg/dl)	1.003	0.995	1.011	0.4676								
Fasting glucose (mg/dl)	1.004	1.000	1.008	0.0348								
UA (mg/dl)	1.123	1.036	1.217	0.0048	1.163	1.047	1.292	0.0049				
CREA(mg/dl)	0.656	0.351	1.227	0.1873								
Homocysteine (umol/dl)	0.961	0.938	0.985	0.0013	0.945	0.915	0.976	0.0006	1.005	1.000	1.011	0.0349
ALKP (mg/dl)	1.014	1.009	1.019	< 0.0001	1.016	1.009	1.023	< 0.0001	0.980	0.947	1.014	0.2471

[#]Suspected NAFLD: ALT ≧ 25 U/l in male; ALT ≧ 17 U/l in female; [※]MetS (-): n = 1492; MetS (+): n = 694.

metabolism. Deregulation of fat metabolism in the fatty liver is associated with overproduction of low density lipoprotein (LDL), very low density lipoprotein (VLDL) and triglycerides. LDL oxidation plays a pivotal role in the development of atherosclerosis. Several lines of evidence suggested that iron is directly involved in the lipid metabolism, and LDL oxidation process may require iron (14, 39, 41). In 1971, Peng and Elson observed increased synthesis of phospholipids in *Tetrahymena pyriformis* grown in medium supplemented with iron (35). Later, it was reported that girls with severe iron deficiency anemia

Table 4. Receiver operating characteristics (ROC) curve analysis for hemoglobin as a predictor of suspected NAFLD in relation to the metabolic syndrome (MetS)

		Hemoglobin cut-point	AUC	Sensitivity (%)	Specificity (%)	Accuracy (%)
All						
	Male	>14.4	0.645	74.0	48.4	63.8
	Female	>13.2	0.640	78.2	49.3	63.7
Mets (+)						
	Male	>14.4	0.665	86.4	42.1	64.2
	Female	>12.9	0.675	69.5	60.2	64.8
Mets (-)						
	Male	>14.0	0.619	69.6	47.9	58.7
	Female	>12.9	0.606	55.5	61.4	58.4

had lower total serum cholesterol and TG concentrations, and that these reduced serum lipid levels returned to normal levels following iron supplementation (14). Recently, it has been shown that LDL is oxidized by iron within the lysosomes of macrophages (41).

The amount of serum ferritin normally reflects the amount of iron stored in the body in healthy individuals, which is about 20-30% of the body iron. However, ferritin is also an acute-phase reactant and elevated serum ferritin levels have been associated with the severity of liver damage in NAFLD subjects (1, 25, 34). Therefore, under conditions of chronic illness, ferritin levels do not reflect the amount of iron stored in the body. Unlike serum ferritin, hemoglobin level is less affected by the presence of acute inflammation. Most of iron within the body is found in hemoglobin within erythrocytes, contributing to about 50% of the body iron. Heme iron is recycled by macrophage following degradation of senescent red blood cells. However, elevated hemoglobin levels may cause increased blood viscosity and decreased blood flow to the liver leading to hypoxia-related liver damages (13, 15, 27, 49). Glycosylation of hemoglobin may also increase the stickiness of the erythrocytes and impair nitric oxide (NO) binding to S-nitrosohemoglobin leading to reduced NO bioavailability in the microvasculature and lower relaxation of hypoxic vessels (5, 12, 23, 30).

Mild hepatic iron overload is frequently observed in NASH and advanced fibrosis and cirrhosis (33). However, the role of hepatic iron in the progression of NASH remains controversial. While some studies found that 20% to 62% of individuals with fatty liver disease had evidence of iron overload, other studies failed to show such a relationship (21, 51). There are two factors, with associated mechanisms, to explain the increased hepatic iron overload: [1] transferrin receptor 1 (TfR1), which takes up iron bound to transferrin; and [2] hepcidin, which controls intestinal iron absorption and regulates the cellular iron export protein ferroportin-1 (Fpn1). Mitsuyoshi *et al.* studied hepatic genes involved in iron metabolism in patients with NAFLD and reported hepatic iron score increased as the stage progressed (36). The stage progression is associated with increased TfR1 genes and decreased hepcidin gene expression (36). Decreased hepatic Fpn1 levels (2) and increased serum hepcidin levels were seen in patients with biopsy proven NAFLD (19, 43).

There are several strengths and limitations in our study. The primary strength of this study was the collection of the nationally representative samples of the general population in Taiwan. The weakness was the definition of the prevalence of NAFLD by elevated ALT levels, and not by the liver biopsy or clinical proven techniques, such as the ultrasonography. Secondly, the exclusion criteria for individuals with chronic liver diseases, which were hepatitis viral infection, hepatocellular carcinoma, and substance abuses, including alcohol or medication, were based on the self-reported health questionnaire. The prevalence of chronic hepatitis B virus (HBV) infection in Taiwan is high (15-20%) (11). Therefore, the presence of the hepatic viral infection may have also led to elevated ALT levels. Morevover, self-reported information on alcohol consumption and substance abuse may be underreported. Finally, validation studies are required to confirm our results.

In conclusion, adults with high hemoglobin levels of 14.4 μg/dl for male and 13.2 μg/dl for female are at the greatest risk for developing abnormal liver function. Hemoglobin test should be considered as a part of clinical evaluation for patients with NAFLD. Furthermore, a lower gender-specific ALT cut-point used in NAFLD diagnosis should be revised for population-based studies.

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