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Original Article

Biomarkers of non-alcoholic fatty liver disease in a Chinese population: a cross-sectional survey

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Abstract: Liver biopsy is the gold standard method for diagnosis of non-alcoholic fatty liver disease (NAFLD), but it is invasive. In this study, relationships between common biochemical indexes and NAFLD were analyzed to explore the clinical value of these non-invasive indicators for clinical diagnosis and treatment. Anthropometric data and biochemical indexes were compared between 204 patients with NAFLD, admitted to the First Affiliated Hospital of Xinjiang Medical University, between January and August 2017, and 521 contemporaneous healthy controls. Male/female ratio, age, height, weight, body mass index (BMI), systolic pressure, diastolic pressure, and proportions with hypertension, diabetes mellitus, or hyperuricemia were higher in the NAFLD group than the control group ($P<0.05$), Alanine aminotransferase, aspartate aminotransferase (AST), γ-glutamyl transpeptidase, uric acid (UA), fasting plasma glucose (FPG), triglycerides (TG), and total cholesterol levels were also higher in the NAFLD group ($P<0.05$). However, serum high-density lipoprotein cholesterol (HDL-C) was lower in the NAFLD group ($P<0.05$). Logistic regression analysis revealed that BMI, AST, UA, FPG, and TG were associated with susceptibility to NAFLD, while HDL-C was protective. Finally, receiver operating characteristic curves of BMI and TG/HDL-C ratios identified patients with NAFLD. Thus, NAFLD was closely associated with glucose and lipid metabolism disorders, along with high FPG, BMI, TG, AST, and UA and low HDL-C. All are risk factors for NAFLD.

Keywords: Biomarkers, non-alcoholic fatty liver disease

Introduction

Non-alcoholic fatty liver disease (NAFLD) is a clinical syndrome characterized by fatty degeneration on liver histology [1]. NAFLD includes a spectrum of chronic liver disease, ranging from hepatic steatosis or fatty liver to non-alcoholic steatohepatitis (NASH), liver fibrosis, liver cirrhosis, and hepatocellular carcinoma (HCC) [2]. Approximately 10%-25% of patients with silent liver disease develop NASH and 5%-8% of these will develop liver cirrhosis within 5 years [3-5]. Furthermore, 12.8% of patients with liver cirrhosis will develop hepatocellular carcinoma (HCC) within 3 years [6]. Prevalence of NAFLD is increasing. It has become the most common chronic disease among Chinese adults in China, more common than other equally important epidemics, including obesity, hypertension, and type 2 diabetes. Moreover, NAFLD is associated with an increase in overall cardiovascular morbidity and mortality [7-10].

Although definitive diagnosis of NAFLD is still histological, the dramatic rise in prevalence and the spectrum of severity suggest that liver biopsy has become impractical for use in all potential cases. Therefore, there has been significant focus on the development and validation of non-invasive biomarkers of NAFLD in recent years [11]. Identification of biomarkers may suggest therapeutic strategies that could be used to prevent inflammation and fibrosis in individuals with fatty liver disease, providing a powerful tool for the monitoring of patients with steatohepatitis. The success of a prevention program will depend on early identification, treatment, and monitoring of high-risk individuals, achieved by assaying many disease-specific biomarkers. Biomarkers would be particularly useful for the quick identification and treatment of patients with fatty liver disease and diagnosis of patients with life-threatening NAFLD and NASH. This would enable classification and staging of the disease using a simple blood test, avoiding the necessity of a liver biopsy [12].

The present study analyzed biochemical indexes in NAFLD patients and healthy controls and

Table 1. Comparison of clinical data between NAFLD and control groups

Item	Control group (n=521)	NAFLD group (n=204)	*P*
Sex (male/female)	258/263	144/60	<0.05
Age (years)	36.27±11.83	38.28±12.25	<0.05
Height (cm)	169.37±8.09	171.48±7.63	<0.05
Weight (kg)	67.50±23.46	79.61±12.52	<0.05
BMI (kg/m^2)	23.75±3.10	26.93±3.12	<0.05
Systolic pressure (mmHg)	120.98±15.10	125.97±15.01	<0.05
Diastolic pressure (mmHg)	72.52±11.90	75.24±12.54	<0.05
Smoke n (%)	130 (25.0)	65 (31.9)	>0.05
Exercise n (%)	350 (67.2)	127 (62.3)	>0.05
Hypertension n (%)	39 (7.5)	38 (18.6)	<0.05
Diabetes mellitus n (%)	10 (1.9)	28 (13.7)	<0.05
Hyperuricemia n (%)	79 (15.2)	49 (24.0)	<0.05

NAFLD: non-alcoholic fatty liver disease; BMI: body mass index.

Table 2. Comparison of biochemical indexes between NAFLD and control groups

Item	Control group (n=521)	NAFLD group (n=204)	*P*
ALT (U/L)	25.61±17.74	35.53±22.04	<0.05
AST (U/L)	23.77±17.55	31.75±23.28	<0.05
GGT (U/L)	29.09±26.50	42.16±33.90	<0.05
BUN (mmol/L)	4.77±1.47	4.98±1.24	>0.05
CRE (μmol/L)	73.31±17.64	74.30±15.17	>0.05
UA (μmol/L)	298.95±90.08	343.44±93.78	<0.05
FPG (mmol/L)	5.08±0.79	5.58±1.62	<0.05
HDL-C (mmol/L)	1.56±0.44	1.34±0.30	<0.05
LDL-C (mmol/L)	2.63±0.85	2.68±0.88	>0.05
TG (mmol/L)	1.35±0.88	2.04±1.40	<0.05
TC (mmol/L)	4.61±1.00	4.83±0.95	<0.05

NAFLD: non-alcoholic fatty liver disease; ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: γ-glutamyl transpeptidase; BUN: blood urea nitrogen; CRE: creatinine; UA: uric acid; FPG: fasting plasma glucose; HDL-C: high-density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol; TG: triglyceride; TC: total cholesterol.

investigated the utility of these non-invasive indices for the diagnosis and treatment of NAFLD.

Materials and methods

Study population

Two hundred and four NAFLD patients from the First Affiliated Hospital of Xinjiang Medical University, between January and August 2017, were recruited for the present study. They included 144 men and 60 women of 20-74 years of age (mean 38.3±12.3 years). NAFLD was diagnosed on the basis of ultrasound criteria listed by the Chinese Medical Association [13]. These criteria included: 1) Diffuse enhancement of near field echo in the hepatic region (stronger than in the kidney and spleen region) and gradual attenuation of the far field echo; 2) Lack of clarity of the intrahepatic lacunae; 3) Mild-to-moderate hepatomegaly with round and blunt borders; and 4) Color Doppler ultrasonography showing a reduction in the blood flow signal or a signal that was difficult to display, but with the distribution of blood flow in the liver being normal. NAFLD was diagnosed if item 1 and any one or more of items 2-4 were present. Hepatic ultrasonographic examinations were performed and conducted by a trained ultrasonographer in a blinded manner.

Patients were excluded from the study if: 1) They consumed too much alcohol (women >70 g/wk, men >140 g/wk); 2) Had other liver diseases, such as viral hepatitis, autoimmune hepatitis, primary biliary cirrhosis, alcoholic liver disease, drug or toxin-associated liver disease, or liver fibrosis; or 3) Had hypertension, coronary atherosclerotic disease, diabetes mellitus, or a malignant tumor.

The healthy control group included 521 healthy individuals that were recruited for the study (258 men and 263 women, aged 18-80 years, with a mean age of 36.3±11.8 years. The study was approved by the Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University and written informed consent was provided by each participant.

Collection of clinical data

Subjects were asked to complete a questionnaire regarding gender, age, smoking habits, alcohol intake, and current and previous medical history. Height, weight, systolic blood pres-

Table 3. Logistic regression analysis of potential risk factors for NAFLD

Item	B	S.E.	Wald	*P*	Exp (B)	95% CI
FPG (mmol/L)	0.275	0.090	9.249	0.002	1.316	1.103-1.571
BMI (Kg/m^2)	0.256	0.037	148.971	0.000	1.292	1.202-1.388
TG (mmol/L)	0.241	0.102	5.620	0.018	1.272	1.043-1.553
AST (U/L)	0.012	0.005	5.454	0.020	1.012	1.002-1.022
UA (μmol/L)	0.003	0.001	5.629	0.018	1.003	1.001-1.006
HDL-C (mmol/L)	-0.816	0.310	6.943	0.008	0.442	0.241-0.811

NAFLD: non-alcoholic fatty liver disease; FPG: fasting plasma glucose; BMI: body mass index; TG: triglyceride; AST: aspartate aminotransferase; UA: uric acid; HDL-C: high-density lipoprotein cholesterol.

Table 4. Predictive value of biochemical indexes for NAFLD, assessed using receiver operating characteristic curves

Item	AUC	95% CI	Cutoff	Sensitivity (%)	Specificity (%)
BMI (Kg/m^2)	0.776	0.739-0.813	27.77	42.600	90.400
TG (mmol/L)	0.715	0.675-0.754	3.46	13.200	96.500
TG/HDL-C	0.733	0.694-0.772	1.58	41.700	86.600
AST (U/L)	0.652	0.608-0.695	60	11.800	96.200
UA (μmol/L)	0.645	0.600-0.690	601	2.000	99.800
FPG (mmol/L)	0.622	0.575-0.669	9.89	3.900	99.600
HDL-C (mmol/L)	0.665	0.622-0.709	0.97	6.900	98.300

NAFLD: non-alcoholic fatty liver disease; BMI: body mass index; TG: triglyceride; AST: aspartate aminotransferase; UA: uric acid; FPG: fasting plasma glucose; HDL-C: high-density lipoprotein cholesterol.

sure, diastolic blood pressure, and body mass index (BMI) were measured.

Specimen collection and analysis

Fasting venous blood was collected from subjects in the early morning (5 mL/sample). Serum was separated and stored at -80°C until analysis. Blood urea nitrogen (BUN), creatinine (CRE), uric acid (UA), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transpeptidase (GGT), fasting plasma glucose (FPG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides (TG), and total cholesterol (TC) were measured using an automatic biochemistry analyzer (DXC800, Beckman Coulter Inc., CA). Effective quality control measures were used and manufacturer instructions were followed.

Statistical analysis

Statistical analyses were conducted using SPSS software version 13.0 for Windows (IBM, Armonk, NY, USA). Continuous data are summarized as mean ± standard deviations, with normality being confirmed using the Kolmogorov-Smirnov test. Independent-samples *t*-test was used for comparisons between two groups and Chi-squared test was used for comparisons between enumerated data. Correlation analysis of risk factors for NAFLD was performed using logistic regression analysis and receiver operating characteristic (ROC) curves were used to assess the performance of variables. $P<0.05$ indicates statistical significance.

Results

Comparison of clinical data

The number of males, age, height, weight, BMI, systolic pressure, diastolic pressure, and proportions of patients with high blood pressure, diabetes, or hyperuricemia in the NAFLD group were higher than the control group (all $P<0.05$). There were no significant differences in smoking or exercise habits between NAFLD and control groups ($P>0.05$) (**Table 1**).

Comparison of blood biochemical indexes

ALT, AST, GGT, UA, FPG, TG, and TC were all significantly higher in the NAFLD group than the control group (all $P<0.05$). However, serum HDL-C concentrations were lower in the NAFLD group than the control group ($P<0.05$). There were no significant differences in serum BUN, CRE, or LDL-C levels between NAFLD and control groups ($P>0.05$) (**Table 2**).

Logistic regression analysis of potential risk factors for NAFLD

A stepwise binary logistic regression model was used to identify independent risk factors for NAFLD. All 23 variables listed in **Tables 1** and **2** were included in the original equation. Six variables remained in the final equation after 17 had been eliminated. These six variables were significantly associated with the presence of

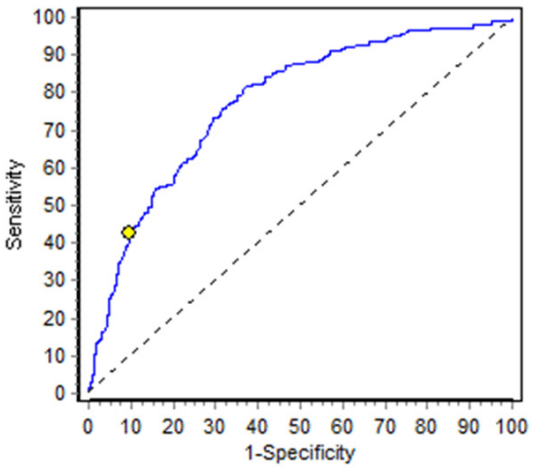

Figure 1. Receiver operating characteristic curve of BMI for NAFLD patients.

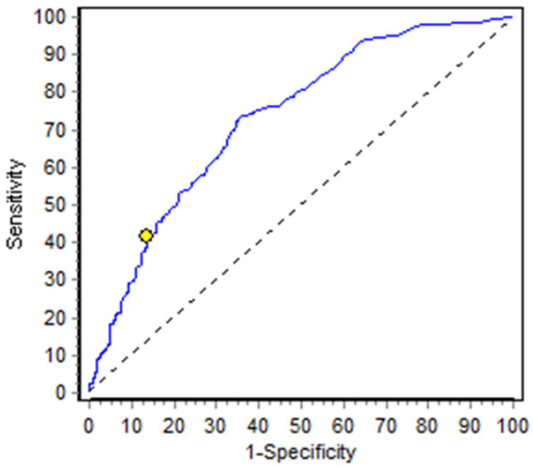

Figure 2. Receiver operating characteristic curve of TG/HDL-C for NAFLD patients.

NAFLD, with FPG, BMI, TG, AST, and UA associated with greater susceptibility and HDL-C being protective (**Table 3**).

Receiver operating characteristic curve analysis of the indexes

This study also plotted receiver operating characteristic (ROC) curves to analyze the predictive value of the above indexes for NAFLD (**Table 4**). ROC curves of BMI and TG/HDL-C ratios were found to effectively identify patients with NAFLD. BMI had an area under the curve of 0.776, sensitivity of 42.6%, and specificity of 90.4%, with a cut-off value of 27.77 (**Figure 1**). BMI could, thus, be used to diagnose NAFLD (P<0.05), with higher BMIs suggesting the greater likelihood of a patient having NAFLD. TG/HDL-C ratios gave an area under the curve of 0.733, sensitivity of 41.7%, and specificity of 86.6%, with a cut-off value of 1.58 (**Figure 2**). TG/HDL-C ratios could, therefore, be used to diagnose NAFLD (P<0.05), with higher TG/HDL-C ratios indicating the greater likelihood of a patient having NAFLD. The value of the other indexes was lower for diagnosis of NAFLD.

Discussion

Fatty liver that does not develop secondary to excessive alcohol consumption is now recognized as the most common cause of chronic asymptomatic liver enzyme elevation in the United States and Europe [14]. It has also become prevalent in China following rapid economic development [15-18], affecting ~5%-24% of the population [19]. Identification of patients that are at risk of developing steatohepatitis that may advance to cirrhosis and are associated with the complications of end-stage liver disease remains a challenge. However, non-invasive biomarkers are being developed to replace liver biopsies. Present results indicate that male/female ratio, age, height, weight, BMI, systolic pressure, diastolic pressure, ALT, AST, GGT, UA, FPG, TG, and TC were higher in the NAFLD group than the control group, while serum HDL-C was lower. Notably, present data did not reveal a difference in LDL-C between the NAFLD and control groups. The reason for this is unclear, although it is possible that a difference in LDL-C between the groups was masked by the numerous other variables being considered. Therefore, multivariate logistic regression analysis was used to study the effects of single factors on NAFLD and to improve the reliability of results, following the exclusion of other confounding factors. Results revealed that FPG, BMI, TG, AST, UA, and HDL-C are independent risk factors for NAFLD.

A previous 8-year follow-up study that investigated prevalence and risk factors for NAFLD in a Chinese population found that 337 participants that did not have NAFLD, at baseline, subsequently developed it. They had greater increases in BMI, serum UA, FPG, and very low-density lipoprotein cholesterol (VLDL), as well as a considerable decrease in high-density lipoprotein cholesterol during the study. In addition, 123 participants that had NAFLD at baseline no longer had it at the end of the follow-up period. These participants had greater reductions in BMI, FPG, TG, TC, low-density lipopro-

tein cholesterol, ALT, AST, and GGT than the other participants [20]. Thus, analysis showed that age, BMI, platelet count, UA, FPG, TG, VLDL cholesterol, HDL cholesterol, ALT, and AST are independent risk factors for NAFLD.

FPG is a major risk factor for NAFLD. In this study, the odds ratio of GLU was 1.32. Furthermore, previous findings have shown that up to 50% of patients with type 2 diabetes mellitus also have NAFLD, whereas the prevalence of NAFLD is as high as 100% in patients with diabetes mellitus complicated with obesity [21].

BMI is a known independent predictor of the degree of hepatic fatty infiltration [22]. In this study, the BMI of the NAFLD group was 26.9± 3.1 kg/m^2, while that of the control group was 23.8±3.1, with an odds ratio of 1.29. Iacobellis et al. [23] reported a study of 69 children with NAFLD, 60% of whom had fibrosis. They found that BMI was the only significant predictor of fibrosis, according to multivariable analysis of simple clinical parameters. In this study, BMI had an odds ratio of 5.85 for predicting the presence of fibrosis. NAFLD has been strongly linked to obesity, with reported prevalences as high as 80% in obese patients, but only 16% in individuals with a normal BMI and no metabolic risk factors [24, 25]. Moreover, many reports have emphasized the importance of weight loss in controlling progression of NAFLD [26, 27].

Metabolic Syndrome (MS) and NAFLD share associations with diabetes, hypertriglyceridemia, and obesity. Because metabolic risk factors are so common in patients with NAFLD, NAFLD may be a hepatic manifestation of MS. Indeed, nearly 90% and 33% of NAFLD patients have at least one feature of MS, respectively. Furthermore, the presence of MS increases the risk of NAFLD by 4-11 times and makes its remission less likely [28, 29]. Present results demonstrate that TG and HDL-C are relevant to NAFLD. High TG was associated with susceptibility to NAFLD, while HDL-C appeared to be protective. Consistently, a previous study also revealed that hypertriglyceridemia and low HDL-cholesterol were present in 62% and 54% of NAFLD patients, respectively [30].

In the present study, AST was also associated with presence of NAFLD, However, ALT was not incorporated into the final regression equation and further studies are needed to confirm the roles of ALT in the development of NAFLD. High liver enzymes are detected in approximately 20% of NAFLD patients [31], reflecting non-specific hepatocellular damage. In NAFLD/NASH, aminotransferase levels may be two to four times higher than the upper limit of the reference range [32]. Elevations in liver enzymes have, therefore, been used as non-invasive indicators of NAFLD [33, 34].

Present data indicates a strong association between high serum UA concentrations and the presence of NAFLD. Hyperuricemia is known to significantly increase the risk of NAFLD and insulin resistance. Wan et al. analyzed the impact of UA on the development of hepatic steatosis and insulin resistance in mice and in two cell models, HepG2 and L02, finding that UA regulates hepatic steatosis and insulin resistance through an NLRP3 inflammasome-dependent mechanism [35]. In addition, Jeffrey examined the association between serum UA and NAFLD in a large population-based study conducted in the United States, finding that higher UA was associated with more severe NAFLD on ultrasonography [36]. Association between serum UA and NAFLD may be the result of one or more of several underlying mechanisms. Multiple studies have shown that when UA enters cells via specific transporters it has pro-inflammatory effects. Intracellularly, it can act as a pro-oxidant, inducing the release of inflammatory mediators and growth factors [37, 38]. Furthermore, UA has been shown to contribute to lipoprotein oxidation and inflammation [39, 40], two “stressors” which are thought to play important roles in the development and progression of NAFLD.

ROC curves were used to determine the predictive values of biochemical indexes for NAFLD. Results showed that BMI and TG/HDL-C ratios could be used to diagnose NAFLD. HDL-C, TG, and other lipid metabolism indexes can be used to predict occurrence of NAFLD. However, the use of HDL-C and TG as early diagnostic indicators of NAFLD has shortcomings, such as large numerical changes, difficulty in grading, and HDL-C and TG measurements not corroborating with one another [41]. Therefore, the TG/HDL-C ratio was used as the main index in the present study, as the range of the index is more

controllable and the number of factors influencing it is smaller.

There were several limitations to the present study. First, biomarkers were not measured on multiple occasions to assess the relationship between changes in these parameters and progression of NAFLD. Second, samples were collected and measured at a single institution. Biomarker values will likely be slightly different in other populations and regions. Third, a lack of information regarding lifestyle and diet may have influenced the relationship between biomarkers and NAFLD. However, despite these limitations, this study provides an extensive and complete dataset regarding the relationship between a wide range of potential risk factors, particularly serum biomarkers, and NAFLD. In conclusion, the present study identified risk factors for NAFLD in a Chinese population that may be useful in risk analysis for the development of NAFLD in patients.

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Disclosure of conflict of interest

None.

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