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Risk Factors for Non-alcoholic Fatty Liver Disease in Chinese Population: A Five-year Follow-up Study

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Abstract

Background: Non-alcoholic Fatty Liver Disease (NAFLD) has become the first liver disease worldwide.
Objectives: This study aimed to explore the prospective risk factors for NAFLD in a large sample of the Chinese population in a five-year follow-up.
Methods: In a cohort study, 1,277 subjects, enrolled in the Second Hospital of Dalian Medical University, were screened initially in 2013 and followed up yearly until 2017. NAFLD was diagnosed based on the ultrasound criteria and the absence of excessive alcohol intake. The follow-up parameters, obtained for 1,165 subjects, included both clinical parameters (body mass index and blood pressure) and biological parameters (fasting plasma glucose (FPG), plasma lipid indices, liver function parameters, and hematological parameters). All statistical analyses were performed using SPSS v20.0 software, and the logistic regression analysis was used to evaluate the risks for incidental and sustained NAFLD.
Results: Individuals with NAFLD at the baseline were more frequently male, old, obese, hypertensive, and diabetic, with hyperuricemia, dyslipidemia, higher liver enzymes, and higher hematological parameters (all $P < 0.001$). Logistic regression analysis showed that BMI and dyslipidemia were the independent predictors of NAFLD (OR = 1.2, 0.1, 5.2, 10.6, and 16.7 for BMI, TC, TG, LDL-C, and HDL-C, respectively, all $P < 0.05$). Moreover, increased systolic and diastolic blood pressure, and the serum transaminases (OR of 0.97, 1.04, and 1.02) were independently associated with sustained NAFLD (all $P < 0.001$).
Conclusions: The present study indicated that increased BMI and dyslipidemia are the potential predictors of NAFLD development and that hypertension and hypertransaminasemia could be the risk factors for NAFLD maintenance. These findings may have practical therapeutic implications.

Keywords: Blood Pressure, Diabetes Mellitus, Follow-up Studies, Glucose, Hypertension, Hyperuricemia, Non-alcoholic Fatty Liver Disease, Obesity, Risk Factors, Transaminases

1. Background

Non-alcoholic Fatty Liver Disease (NAFLD) is a syndrome characterized by hepatic pathological features similar to alcoholic fatty liver disease in the absence of excessive alcohol intake. It is the most frequent chronic liver disease in developed countries (1). Its prevalence in the general population ranges from 17% to 46% (2). NAFLD is a potentially severe disease, as it can progress to aggressive forms of non-alcoholic steatohepatitis (NASH), with the risk of cirrhosis, hepatocellular failure, and hepatocellular carcinoma (3). Moreover, NAFLD is a recognized risk factor for metabolic syndrome, type 2 diabetes, and cardiovascular disease (4).

It is critical to identify which group of patients is at the highest risk of NAFLD to implement targeted therapeutic interventions. Age, gender, obesity, over nutrition, and insulin resistance are widely accepted to be associated with the development of NAFLD. However, the direct relationships between age/gender and susceptibility to NAFLD remain unsettled (5). Dalian is a coastal city in the northeast of China, where seafood and a high-fat diet have become a kind of alimentary habit. All previous studies were based on data obtained from relatively small population samples with short follow-up duration, which limited the evaluation of NAFLD prevalence and incidence in the general population.

2. Objectives

The present study is a five-year follow-up report of 1,165 people in Dalian, China, aimed at to evaluate the preva-

lence and facilitating factors of NAFLD, as well as the risk factors for the development and evolution of NAFLD.

3. Methods

3.1. Subjects

The cohort study population comprised randomly selected 1,277 individuals who had continuously benefited from an annual health check-up at the Second Hospital of Dalian Medical University. The exclusion criteria were (1) excess alcohol consumption (more than 140 g per week), (2) markers of hepatitis B virus (HBV) infection (HBsAg) and hepatitis C virus (HCV) infection (antiHBC-Ab), (3) drug-induced hepatitis, autoimmune hepatitis, hyperthyroidism, or cancer, and (4) cardiovascular disease. Accordingly, 112 individuals were excluded. Therefore, 1,165 individuals (621 males and 544 females) were followed up for five years until the final evaluation point in 2017 (Figure 1). This study was approved by the Ethics Committee of the Second Hospital of Dalian Medical University. Written informed consent was obtained from all participants (or their legal guardians) before study enrollment.

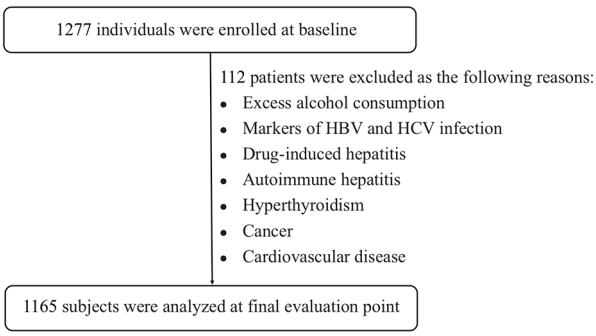

Figure 1. The flow diagram of the study

This sample size was calculated using Sample Size Calculator software (https://www.surveysystem.com/sscalc.htm#one). The confidence level and confidence intervals were 4 and 99%. For the population, we chose 1000 so that the sample size was 510. Two observers examined the medical records of patients with the kappa coefficient of 0.89.

3.2. Clinical and Anthropometric Measurements

Physical examination was performed in the morning for body mass index (BMI) and blood pressure. BMI was measured by Inbody 220 (Korean Biospace Corporation). Blood pressure was measured by the Omron HEM-906 wrist blood pressure monitor. The equipment was calibrated every three months by the maintainers. Hypertension was diagnosed when a patient was under antihypertensive medication or had systolic blood pressure (SBP) of $\geq$ 140 mmHg or diastolic blood pressure (DBP) of $\geq$ 90 mmHg.

3.3. Biomedical Measurements

After an overnight fast, a venous blood sample of 3 milliliters (mL) was obtained from each participant for biochemical analyses without freezing. An automatic analyzer (Hitachi Inc., Japan) was used to measure red blood cell (RBC) count, hemoglobin (HGB), platelet (PLT) count, white blood cell (WBC) count, serum uric acid (SUA), fasting blood glucose (FBG), triglycerides (TG), total cholesterol (TC), low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, total bilirubin (TB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyl transpeptidase (GGT) according to the standard methods.

Diabetes mellitus was diagnosed if the patient had an FBG level of $\geq$ 7.0 mmol/L or was treated with antidiabetic medications or insulin. Hypercholesterolemia was diagnosed if the patient had a TC level of $\geq$ 6.22 mmol/L. Hypertriglyceridemia was diagnosed based on the TG level of $\geq$ 2.26 mmol/L. High LDL-C was defined by levels $\geq$ 4.14 mmol/L and low HDL-C by levels $\leq$ 1.04 mmol/L. According to the 1997 diagnostic criteria of the American Rheumatism Association, hyperuricemia was defined by the levels of $\geq$ 420 mmol/L in men and $\geq$ 357 mmol/L in women.

3.4. Ultrasonography

Ultrasonography was performed to diagnose NAFLD following the Assessment and Management Guidelines of Nonalcoholic Fatty Liver Disease in Asia and the Pacific Region (5). Fatty liver was defined as "diffuse" if it met two of the following three criteria: (1) diffuse enhancement of near-field echo in the hepatic region, with the echo stronger than that in the kidneys, (2) lack of clear visualization of intrahepatic ductal structures, and (3) gradual attenuation of the far-field echo in the hepatic region.

3.5. Statistical Analyses

Statistical analyses were performed using IBM SPSS Statistics for Windows, version 20.0 (IBM Corp., Armonk, N.Y., USA). The normality of the data was checked before data analysis through the one-sample Kolmogorov-Smirnov test. The normal data were displayed as means $\pm$ SD and abnormal data as median (interquartile range). Furthermore, the Mann-Whitney U test and Student's *t*-test were run to analyze the abnormal and normal data, respectively. Categorical variables were compared between

groups using the chi-squared test. The potential risk factors for NAFLD were analyzed using the logistic regression model. All reported P values were two-tailed, and the values below 0.05 were considered statistically significant.

4. Results

4.1. Baseline Characteristics

There were 621 (53.5%) men among the 1,165 enrolled participants with an overall mean (± SD) age of 38.3 ± 9.0 years. The ultrasound NAFLD criteria were met by 363 subjects (31.2%; 303 men and 60 women). Clinical and biochemical parameters of the whole cohort are shown in Table 1. The prevalence of NAFLD was 48.8% and 11.0% in men and women, respectively. NAFLD patients were older than non-NAFLD individuals ($P < 0.01$). The NAFLD patients had significantly higher BMI, SBP, DBP, ALT, AST, GGT, TC, TG, LDL-C, and FBG and lower HDL-C levels than those without NAFLD (all $P < 0.01$). Moreover, blood cell parameters (such as RBC count, WBC count, and HGB concentration) were all higher in the NAFLD group (all $P < 0.001$). The overall prevalence of hypertension, diabetes mellitus, hypercholesterolemia, hypertriglyceridemia, high LDL cholesterol, low HDL cholesterol, and hyperuricemia were also higher in the NAFLD group.

4.2. Development of NAFLD After a Five-Year Follow-up

After five years of follow-up, 10.1% (81/802) of the subjects without NAFLD at the start of the study developed NAFLD. Subjects who developed NAFLD had significantly higher abnormal values than those who maintained good health in most parameters at baseline, especially for BMI, SBP, DBP, FBG, TG, HDL-C, SUA, TB, WBC, RBC, HGB, ALT, AST, and GGT (Table 2).

When comparing the two groups in terms of changes in all parameters from baseline to the end of the follow-up, subjects who developed NAFLD showed greater changes in BMI, FBG, TG, SUA, RBC, and HGB (Table 3). When exploring the independent risk factors associated with NAFLD development by logistic regression, five variables remained in the final equation, including BMI ($P < 0.01$, OR = 1.2, 95% CI: 1.1 - 1.3), TC ($P < 0.01$, OR = 0.1, 95% CI: 0.0 - 0.5), TG ($P < 0.01$, OR = 5.2, 95% CI: 2.2 - 12.2), LDL-C ($P < 0.05$, OR = 10.6, 95% CI: 1.6 - 67.8), and HDL-C ($P < 0.05$, OR = 16.7, 95% CI: 1.6 - 175.0).

4.3. NAFLD Improvement After a Five-Year Follow-up

The total percentage of improved NAFLD cases was 31.0% (115/363). Subjects with improved NAFLD had relatively lower TG, SUA, WBC, ALT, and AST levels and higher HDL-C levels at baseline than the subjects with maintained NAFLD (Table 4).

When comparing these two groups in terms of changes from baseline to the endpoint, we observed that BMI, FBG, TG, RBC, and HGB levels were much higher and HDL-C was lower in the sustained NAFLD group (Table 5).

To identify variables effective in predicting the maintenance of NAFLD, a binary logistic regression analysis was performed. The predictors of NAFLD maintenance were SBP ($P = 0.03$, OR = 0.97, 95% CI: 0.95 - 1.0), DBP ($P = 0.04$, OR = 1.04, 95% CI: 1.0 - 1.07), and ALT ($P = 0.04$, OR = 1.02, 95% CI: 1.0 - 1.05).

5. Discussion

Non-alcoholic fatty liver disease (NAFLD), which is the main cause of chronic liver disease in the United States, is increasingly prevalent worldwide (6). NAFLD prevalence in Western countries has been estimated between 20% and 30% (7) while it ranges from 17% to 46% worldwide according to the population (8). In the present health check study, we demonstrated that the prevalence of NAFLD was 31.2% in 2013, with a much higher prevalence in older people.

In agreement with other studies (9), our results indicated a significant difference in the NAFLD prevalence between men and women. This difference might be related to the protective role of estrogens in women (2). Differences in sex hormone levels are likely to explain the gender differences in the amount and distribution of body fat (10). Men usually store fat in the abdomen, whereas women store it in the subcutaneous tissue (11), and visceral adipose tissue is known to play a role in NAFLD. One important finding of our study is that NAFLD does not always develop unfavorably, allowing us to explore further the main factors underlying the development and maintenance of NAFLD.

Obesity is well known to correlate with both NAFLD prevalence and severity. In our study, the baseline BMI levels were higher in subjects who developed NAFLD at the end of the study. Moreover, BMI changes were significantly higher in those individuals who developed NAFLD than in those who remained in good health, corresponding to an odd of 1.2 for NAFLD development.

When focusing on the subjects with NAFLD at baseline, those who maintained their NAFLD status had both higher baseline and evolving BMI values after five years of follow-up. In contrast, BMI decreased over time in those subjects who had NAFLD improvement. Previous reports also concluded that moderate weight loss was associated with improved fatty liver and other pathological features (12). Recent studies point out the importance of controlling body weight for both obese and non-obese subjects so that the risk of NAFLD is prevented or decreased (13).

It is well known that hypertension is associated with the development of NAFLD. In our follow-up study, we

Table 1. Baseline Characteristics of the Subjects with and Without NAFLD

Variables	NAFLD (N = 363)	Non-NAFLD (N = 802)	χ^2/t/z Value	P Value
Continuous data				
Age, y	41.5 ± 8.2	36.9 ± 9.0	-8.2	< 0.01
BMI, kg/m^2	27.1 ± 2.8	22.2 ± 2.7	-28.3	< 0.01
SBP, mmHg	129.2 ± 15.5	114. 5 ± 14.9	-15.4	< 0.01
DBP, mmHg	79.8 ± 12.2	69.4 ± 11.2	-14.3	< 0.01
FBG, mmol/L	5.8 ± 1.2	5.3 ± 0.5	-11. 5	< 0.01
TG, mmol/L	4. 9 ± 0.8	4.5 ± 0.7	-17.9	< 0.01
TC, mmol/L	1.9 ± 1.2	1.0 ± 0.6	-16.7	< 0.01
HDL-C, mmol/L	1.1 ± 0.2	1.3 ± 0.3	15.0	< 0.01
LDL-C, mmol/L	3.1 ± 0.7	2.7 ± 0. 7	-8.4	< 0.01
SUA, μmol/L	384.7 ± 88.8	301.1 ± 78.4	-16.2	< 0.01
TB, μmol/L	15.3 ± 6.5	14.0 ± 5.9	-3.2	< 0.01
RBC, $\times 10^{12}$/L	5.1 (4.8 ~ 5.3)	4.7 (4.4 ~ 5.0)	-13.0	< 0.01
HGB, g/L	153.3 (147.0 ~ 162.0)	139.6 (130.0 ~ 151.0)	-14.0	< 0.01
WBC, $\times 10^9$/L	6.9 ± 1.6	6.1 ± 1.5	-8.2	< 0.01
PLT, $\times 10^9$/L	221.5 ± 47.4	226.8 ± 52.7	1.6	0.11
ALT, mmol/L	40.1 (24.0 ~ 48.0)	19.8 (12.0 ~ 23.0)	-18.1	< 0.01
AST, mmol/L	31.0 (23.0 ~ 35.0)	24.2 (19.0 ~ 27.0)	-12.6	< 0.01
GGT, mmol/L	46.3 (26.0 ~ 56.0)	23.7 (13.0 ~ 25.0)	-18.0	< 0.01
Categorical data				
Sex: males, No. (%)	303 (83.5)	318 (39.7)	192.8	< 0.01
Diabetes mellitus, No. (%)	35 (9.6)	8 (1.0)	52.5	< 0.01
Hypertension, No. (%)	104 (28.7)	59 (7.4)	94.2	< 0.01
Overweight, No. (%)	327 (90.1)	197 (24.6)	433.5	< 0.01
Hypercholesterolemia, No. (%)	23 (6.3)	14 (1.7)	17.1	< 0.01
Hypertriglyceridemia, No. (%)	91 (25.1)	31 (3.9)	119.8	< 0.01
High LDL cholesterol, No. (%)	30 (8.3)	21 (2.6)	19.0	< 0.01
Low HDL cholesterol, No. (%)	163 (44.9)	129 (16.1)	110.5	< 0.01
Hyperuricemia, No. (%)	123 (33.9)	93 (11.6)	82.2	< 0.01

Abbreviations: ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; BMI, Body Mass Index; DBP, Diastolic Blood Pressure; FBG, Fasting Blood Glucose; GGT, Gammaglutamyl Transpeptidase; HDL, High-Density Lipoprotein; HGb, Hemoglobin; LDL, Low-Density Lipoprotein; PLT, Platelet; RBC, Red Blood Cells; SBP, Systolic Blood Pressure; SUA, Serum Uric Acid; TB, Total Bilirubin; TC, Total Cholesterol; TG, Triglycerides; WBC, White Blood Cells

found that newly developed NAFLD subjects had higher levels of SBP and DBP at baseline. Moreover, our data showed that SBP and DBP were risk factors independently associated with NAFLD maintenance.

Independent of BMI levels (1), type 2 diabetes is frequent among NAFLD subjects (14, 15). Our study showed that not only the baseline but also the increasing levels of FBG were much higher in maintained NAFLD subjects. Moreover, our study showed that NAFLD maintenance was associated with more FBG increases than NAFLD improvement was although the baseline FBG levels were not significantly different between the two groups. Taken together, these findings highlight the importance of diabetes for NAFLD progression.

Dyslipidemia is frequent in individuals with NAFLD. NAFLD dyslipidemia is characterized by high SC, TG, and LDL-C and low HDL-C. In our study, we found that not only the newly developed but also the maintained NAFLD subjects exhibited higher TG levels and lower HDL-C levels. Moreover, multiple logistic regression analysis showed

Table 2. Comparison of Baseline Variables Between the Subjects Who Maintained Good Health and Those Who Developed NAFLD at the Endpoint

Variables	Healthy (N = 721)	New NAFLD (N = 81)	t/z Value	P Value
Age, y	36.6 (29.0 ~ 43.0)	39.5 (31.0 ~ 45.5)	-2.5	0.01
BMI, kg/m^2	22.0 ± 2.7	24.1 ± 2.6	-6.8	< 0.01
SBP, mmHg	113.9 ± 14.6	119.6 ± 16.6	-3.3	< 0.01
DBP, mmHg	68. 9 ± 11.0	74.2 ± 11. 6	-4.1	< 0.01
FBG, mmol/L	5.3 ± 0.5	5.4 ± 0.5	-2. 6	0.01
TG, mmol/L	1.0 (0.7 ~ 1.1)	1.5 (0.9 ~ 1.8)	-7.5	< 0.01
TC, mmol/L	4.5 ± 0.7	4.6 ± 0.7	-1.2	0.24
HDL-C, mmol/L	1.3 ± 0.3	1.2 ± 0.3	4.5	< 0.01
LDL-C, mmol/L	2.7 ± 0. 7	2. 8 ± 0.7	-1.1	0.29
SUA, μmol/L	296.5 (239.5 ~ 344.0)	342.4 (289.5 ~ 400.5)	-5.2	< 0.01
TB, μmol/L	14.1 ± 6.0	13.4 ± 4.7	1.1	0.30
RBC, $\times 10^{12}$/L	4. 7 (4.4 ~ 5.0)	4. 9 (4.5 ~ 5.3)	-4.2	< 0.01
HGB, g/L	138.7 ± 15.7	147.2 ± 16.1	-4.6	< 0.01
WBC, $\times 10^9$/L	6.0 (5.1 ~ 6.8)	6.7 (5.2 ~ 7.4)	-2.8	< 0.01
PLT, $\times 10^9$/L	226.5 (194.0 ~ 256.0)	229.4 (198.0 ~ 249.5)	-0.3	0.78
ALT, mmol/L	18.8 (12.0 ~ 22.0)	29.0 (16.0 ~ 36.0)	-5.9	< 0.01
AST, mmol/L	23.8 (19.0 ~ 26.0)	28.3 (21.0 ~ 31.5)	-4.5	< 0.01
GGT, mmol/L	22.2 ± 22.3	37.3 ± 37.6	-5.3	< 0.01

Abbreviations: ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; BMI, Body Mass Index; DBP, Diastolic Blood Pressure; FBG, Fasting Blood Glucose; GGT, Gammaglutamyl Transpeptidase; HDL-C, High-Density Lipoprotein Cholesterol; HGb, Hemoglobin; LDL-C, Low-Density Lipoprotein Cholesterol; PLT, Platelet; RBC, Red Blood Cells; SBP, Systolic Blood Pressure; SUA, Serum Uric Acid; TB, Total Bilirubin; TC, Total Cholesterol; TG, Triglycerides; WBC, White Blood Cells

Table 3. Comparison of the Parameter Changes Between the Subjects Who Maintained Good Health and Those Who Developed NAFLD at the Endpoint

Variables	Healthy Subjects (N = 721)	Subjects Developing NAFLD (N = 81)	t Value	P Value
BMI, kg/m^2	0.5 ± 1.3	1.6 ± 1.7	-7.3	< 0.01
SBP, mmHg	7.1 ± 11.4	9.3 ± 13.6	-1.6	0.11
DBP, mmHg	4.3 ± 8.8	5.7 ± 10.0	-1.3	0.18
FBG, mmol/L	0.1 ± 0.6	0.4 ± 0.7	-3.3	< 0.01
TG, mmol/L	0.1 ± 0.5	0.3 ± 0.7	-2.1	0.04
TC, mmol/L	0.2 ± 0.6	0.4 ± 0.7	-2.0	0.05
HDL-C, mmol/L	0.1 ± 0.2	0.1 ± 0.2	1.7	0.10
LDL-C, mmol/L	-0.3 ± 0.5	-0.1 ± 0.5	-2.4	0.02
SUA, μmol/	0.6 ± 46.0	17.5 ± 61.9	-3.0	< 0.01
TB, μmol/L)	0.3 ± 4.9	0.3 ± 4.7	0.0	0.99
RBC, $\times 10^{12}$/L	0.1 ± 0.2	0.2 ± 0.3	-3.2	< 0.01
HGB, g/L	0.6 ± 8.8	3.8 ± 8.1	-3.0	< 0.01
WBC, $\times 10^9$/L	-0.3 ± 1.2	-0.2 ± 1.3	-0.5	0.59
PLT, $\times 10^9$/L	16.2 ± 33.9	16.7 ± 32.8	-0.1	0.91
ALT, mmol/L	-0.6 ± 13.1	0.8 ± 24.3	-0.8	0.41
AST, mmol/L	-4.3 ± 8.3	-5.4 ± 10.7	1.1	0.28
GGT, mmol/L	-4.8 ± 12.3	-6.1 ± 31.7	0.7	0.47

Abbreviations: ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; BMI, Body Mass Index; DBP, Diastolic Blood Pressure; FBG, Fasting Blood Glucose; GGT, Gammaglutamyl Transpeptidase; HDL-C, High-Density Lipoprotein Cholesterol; HGb, Hemoglobin; LDL-C, Low-Density Lipoprotein Cholesterol; PLT, Platelet; RBC, Red Blood Cells; SBP, Systolic Blood Pressure; SUA, Serum Uric Acid; TB, Total Bilirubin; TC, Total Cholesterol; TG, Triglycerides; WBC, White Blood Cells

that TC, TG, LDL-C, and HDL-C were independent risk factors for newly developed NAFLD (OR = 0.07, 5.21, 10.53, and 16.69, respectively). These results are in agreement with the cohort study by Xu et al. (16). Therefore, lifestyle changes (e.g., weight loss and enhanced physical activity) are critical for counteracting dyslipidemia in NAFLD.

The close relationship between SUA and NAFLD has already been emphasized. A meta-analysis indicated that the elevated SUA levels were associated with NAFL occurrence independently of age, gender, obesity, and metabolic

Table 4. Comparison of the Baseline Variables Between the Subjects with Maintained NAFLD and Those with Improved NAFLD at the Endpoint

Variables	Improved NAFLD (N = 115)	Maintained NAFLD (N = 248)	t/z Value	P Value
Age, y	42.3 (37.0 ~ 49.0)	41.1 (36 ~ 48)	-1.0	0.31
BMI, kg/m^2	26.7 ± 2.6	27.3 ± 2.9	-1.8	0.07
SBP, mmHg	129.3 ± 16.9	129.1 ± 14.8	0.1	0.90
DBP, mmHg	78.7 ± 12.4	80.4 ± 12.1	-1.2	0.24
FBG, mmol/L	5.0 (5.2 ~ 5.9)	5. 9 (5.3 ~ 6.1)	-1.2	0.22
TG, mmol/L	1.7 (1.1 ~ 1.9)	2.0 (1.3 ~ 2.4)	-2.7	0.01
TC, mmol/L	4.8 ± 0.8	4.9 ± 0.9	-1.0	0.33
HDL-C, mmol/L	1.1 ± 0.2	1.1 ± 0.2	2.2	0.03
LDL-C, mmol/L	3.1 ± 0.7	3.1 ± 0.8	-0.3	0.78
SUA, μmol/L	369.8 (318.0 ~ 414.0)	391.6 (330.0 ~ 442.5)	-2.3	0.02
TB, μmol/L	16.2 ± 6.7	14.9 ± 6.4	1.8	0.08
RBC, $\times 10^{12}$/L	5.0 ± 0.4	5.1 ± 0.4	-1.0	0.34
HGB, g/L	152.7 (147.0 ~ 162.0)	153.6 (147.3 ~ 162.0)	-0.5	0.65
WBC, $\times 10^9$/L	6.6 (5.6 ~ 7.4)	7.0 (5.8 ~ 7.9)	-2.5	0.01
PLT, $\times 10^9$/L	220.3 (189.0 ~ 242.0)	222.1 (187.0 ~ 250.0)	-0.2	0.83
ALT, mmol/L	32.1 (19.0 ~ 38.0)	43.9 (26.0 ~ 51.8)	-5.0	<0.01
AST, mmol/L	28.1 (22.0 ~ 32.0)	32.3 (24.0 ~ 38.0)	-3.6	<0.01
GGT, mmol/L	41.8 (24.0 ~ 53.0)	48.4 (27.3 ~ 59.0)	-2.0	0.06

Abbreviations: ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; BMI, Body Mass Index; DBP, Diastolic Blood Pressure; FBG, Fasting Blood Glucose; GGT, Gammaglutamyl Transpeptidase; HDL-C, High-Density Lipoprotein Cholesterol; HGb, Hemoglobin; LDL-C, Low-Density Lipoprotein Cholesterol; PLT, Platelet; RBC, Red Blood Cells; SBP, Systolic Blood Pressure; SUA, Serum Uric Acid; TB, Total Bilirubin; TC, Total Cholesterol; TG, Triglycerides; WBC, White Blood Cells

syndrome (17). Zhou et al. showed that elevated SUA concentrations were inversely correlated with NAFLD remission, suggesting that lowering SUA levels may reduce the risk of NAFLD (18). Our study also indicated that SUA was an independent risk factor for NAFLD development and maintenance. Insulin resistance, hyperuricemia-related endothelial dysfunction, oxidative stress, and inflammatory lesions could be involved in the relationship between SUA and NAFLD (19-22).

Previous studies reported that RBC counts and HBG were risk markers for NAFLD (23). In our study, the RBC count and HBG were positively associated with NAFLD prevalence, and these parameters were higher in NAFLD subjects than in non-NAFLD subjects. Moreover, increased RBC count was independently associated with the risk of NAFLD (24). Feng et al., in a study of 2000 subjects, reported that both lean and overweight NAFLD patients had higher RBC counts (25). One possible mechanism could be compensatory erythropoiesis of metabolic and detoxification functions related to defects in the fatty liver. Whether iron overload reported in NAFLD (26) could have a link with dyserythropoiesis remains to be elucidated.

In this study, the baseline levels of liver enzymes were associated with the development and maintenance of NAFLD. However, the changes in liver enzymes during the follow-up were not different between the groups. Our results also showed that ALT was an independent risk factor for sustained NAFLD. Increased ALT activity has been associated with visceral fat, hepatic steatosis, inflammation, and fibrosis (27).

Due to the complex environmental and metabolic factors that could affect the etiology of NAFLD and its progression, further studies are warranted to demonstrate the potential factors underlying the progression of NAFLD. In this study, much effort was made to follow the patients over five years and figure out the main risk factors related to NAFLD. It is essential to understand the clinical factors underlying NAFLD development for ensuring appropriate prevention and treatment of this increasing population of patients. However, there are several limitations to this study. First, NAFLD diagnosis was made by ultrasonography examination, which is known to lack sensitivity. However, ultrasonography is widely used in population-based studies and is considered to have a high diagnostic value in NAFLD (2, 28). Second, our study sample, although consequent, was not large enough to explore the association between NAFLD and the risk factors in depth. Finally, it has been shown that insulin resistance is pivotal to the pro-

Table 5. Comparison of the Changes in All Parameters Between the Subjects with Improved and Those with Maintained NAFLD at the Endpoint

Variables	Remitted NAFLD (N = 115)	Sustained NAFLD (N = 248)	t Value	P Value
BMI, kg/m^2	-0.7 ± 1. 8	0. 5 ± 1.3	-7.1	< 0.01
SBP, mmHg	2. 9 ± 13.7	3.7 ± 13.1	-0.5	0.61
DBP, mmHg	3.1 ± 9.3	2.7 ± 10.3	0.3	0.74
FBG, mmol/L	0.3 ± 0.7	0.6 ± 1.3	-2.4	0.02
TG, mmol/L	-0.1 ± 0.8	0.2 ± 1.0	-2.3	0.02
TC, mmol/L	-0.0 ± 0.8	0.1 ± 0.7	-0.8	0.40
HDL-C, mmol/L	0.2 ± 0.2	0.1 ± 0.2	2.1	0.04
LDL-C, mmol/L	-0.4 ± 0.6	-0.4 ± 0.6	-0.3	0.76
SUA, μmol/L	-2.1 ± 65.8	0.5 ± 62.6	-0.4	0.72
TB, μmol/L	1.3 ± 5.1	0.6 ± 4.5	1.4	0.18
RBC, $\times 10^{12}$/L	0.0 ± 0.2	0.1 ± 0.2	-3.0	< 0.01
HGB, g/L	-0.6 ± 8.9	1.2 ± 7.5	-2.0	0.04
WBC, $\times 10^{9}$/L	-0.2 ± 1.2	-0.2 ± 1.2	0.4	0.68
PLT, $\times 10^{9}$/L	10.3 ± 29.4	13. 9 ± 26.4	-1.2	0.25
ALT, mmol/L	-6.6 ± 24. 9	-6. 7 ± 25.3	0.0	0.97
AST, mmol/L	-6.1 ± 11.0	-7.2 ± 13.2	0.8	0.43
GGT, mmol/L	-12.7 ± 18.1	-9.9 ± 21.6	-1.2	0.24

Abbreviations: ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; BMI, Body Mass Index; DBP, Diastolic Blood Pressure; FBG, Fasting Blood Glucose; GGT, Gammaglutamyl Transpeptidase; HDL-C, High-Density Lipoprotein Cholesterol; HGb, Hemoglobin; LDL-C, Low-Density Lipoprotein Cholesterol; PLT, Platelet; RBC, Red Blood Cells; SBP, Systolic Blood Pressure; SUA, Serum Uric Acid; TB, Total Bilirubin; TC, Total Cholesterol; TG, Triglycerides; WBC, White Blood Cells

gression of NAFLD (29), but we did not include this factor in our study. Moreover, the roles of medications and dietary habits in NAFLD development and regression were not considered in the present study, and they deserve further investigation.

5.1. Conclusions

In conclusion, the current study found that BMI, TC, TG, LDL-C, and HDL-C were significant predictors of NAFLD development and that SBP, DBP, and ALT were risk factors associated with NAFLD maintenance.

These results have practical implications by strongly suggesting that weight loss, adequate management of hyperlipidemia, hypertension, and liver inflammation may be beneficial not only for attenuating the disease severity and progression but also for improving established nonalcoholic fatty liver disease.

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Footnotes

Authors' Contribution: Study concept and design: Bin Hu. Acquisition and statistic analysis of the data: Hui Zhao. Drafting of the manuscript and critical revision of the manuscript for important intellectual content: Bin Hu and Hui Zhao.

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