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ORIGINAL RESEARCH

Predictors of High Cardiovascular Risk Among Nonobese Patients with Type 2 Diabetes and Non-Alcoholic Fatty Liver Disease in a Chinese Population

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Purpose: This study aims to investigate cardiovascular risk factors in nonobese patients with type 2 diabetes (T2DM) and non-alcoholic fatty liver disease (NAFLD) and to determine whether they might be used to predict high-risk individuals effectively.

Patients and Methods: This cross-sectional study included 245 nonobese patients with T2DM who underwent FibroTouch in the National Metabolic Management Center of the Affiliated Changzhou No. 2 People's Hospital of Nanjing Medical University from January 2021 to December 2022. All individuals were divided into NAFLD and non-NAFLD groups. Patients with NAFLD were further grouped by UAP tertiles (T1, T2 and T3). We created a Cardiovascular Score (total scale: 0–5 points; ≥3 points was defined as high-risk individual) based on baPWV, carotid ultrasound, and urinary microalbumin creatinine ratio (UA/CR) to assess the risk of cardiovascular disease in non-obese T2DM patients with NAFLD. Risk factors were evaluated using univariate and multivariate analysis. The performance of risk factors was compared according to the area under the receiver operating characteristic (ROC) curve.

Results: Atherogenic index of plasma (AIP), atherosclerosis index (AI), prevalence of hypertension, body mass index (BMI) and homeostatic model assessment of insulin resistance (HOMA-IR) were higher in the NAFLD group compared to the non-NAFLD group. In T3 group, AIP, AI, BMI and HOMA-IR were higher than those of T1 group. Multivariate logistic regression showed that age, systolic blood pressure, low-density lipoprotein-cholesterol (LDL-C) and AIP were risk factors for cardiovascular disease among nonobese patients with T2DM and NAFLD. The area under the ROC curve for age, systolic blood pressure, LDL-C and AIP were 0.705, 0.688, 0.738 and 0.642, respectively. The area under the ROC curve was 0.895 when combining them.

Conclusion: Age, systolic blood pressure, AIP and LDL-C are all independent risk factors for cardiovascular disease in non-obese individuals with T2DM and NAFLD, which can be combined to identify high-risk populations and carry out intervention.

Keywords: T2DM, non-obese, NAFLD, cardiovascular disease

Introduction

Non-alcoholic fatty liver disease (NAFLD) is a chronic hepatic disorder characterized by the presence of ≥5% steatosis in the liver, without a history of excessive alcohol consumption and excluding other hepatopathies.[1] A meta-analysis revealed that the global prevalence of NAFLD was 25.24%.[2] Although NAFLD is more prevalent among individuals with obesity, it should not be underestimated in non-obese populations. Epidemiological evidence suggests that approximately 40% of individuals diagnosed with NAFLD are non-obese.[3] The prevalence of non-obese NAFLD varies from 5% to 26% in Asian populations and ranges from 7% to 20% in Western populations.[4]

Studies have demonstrated that NAFLD remains an independent risk factor for the development of T2DM, cardiovascular disease, and chronic kidney disease even after adjusting for metabolic risk factors such as BMI and waist

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circumference.[5] Furthermore, NAFLD was associated with a significantly elevated risk of all-cause and cardiovascular mortality in non-obese individuals.[2] A study revealed that the overall mortality rate for non-obese NAFLD patients was as high as 12.1 per 1000 person-years.[3] Interestingly, recent research has demonstrated that non-obese NAFLD patients were found to be at 1.96 times higher risk of all-cause mortality compared to obese NAFLD patients, with cardiovascular disease emerging as the primary cause of death among individuals affected by NAFLD.[6,7] Additionally, diabetes independently contributes to the increased risk of developing cardiovascular disease, which represents the leading cause of mortality in diabetic patients.[4] A large study showed that individuals with T2DM and NAFLD had 2.01 times higher risk of developing cardiovascular diseases compared to those without NAFLD.[8] NAFLD increases the risk of cardiovascular disease by several mechanisms. NAFLD is associated with hepatic insulin resistance, which causes metabolic disorders of apolipoprotein B (apoB), and increases de novo synthesis of lipids in the liver, resulting in an increase in very low-density lipoprotein (VLDL) production. Rising levels of circulating VLDL can lead to an increase in low-density lipoprotein (LDL) production and accelerate atherosclerosis.[9,10] Additionally, elevated levels of inflammation in NAFLD can lead to vascular endothelial damage, which may facilitate arterial plaque formation and heighten cardiovascular risks.[11]

In summary, both NAFLD and T2DM are independent risk factors for cardiovascular disease, and the risk of cardiovascular disease is further increased by having both diseases simultaneously. While previous studies predominantly focused on exploring the association between obese NAFLD or obese T2DM and cardiovascular disease, limited research exists regarding cardiovascular risk factors in non-obese individuals with both T2DM and NAFLD. Therefore, this study aims to investigate these specific risk factors for cardiovascular disease among non-obese patients diagnosed with both T2DM and NAFLD while providing valuable insights into prevention strategies and treatment approaches targeting this population.

Materials and Methods

Study Population

Patients who underwent FibroTouch in the National Metabolic Management Center of the Affiliated Changzhou No. 2 People's Hospital of Nanjing Medical University from January 2021 to December 2022 were enrolled. A total of 3000 participants were recruited and selected on the basis of the following exclusion criteria: (1) Body mass index (BMI) ≥ 25 kg/m^2; (2) failed to meet the diagnostic criteria for T2DM in the 2016 ADA guidelines; (3) excess alcohol consumption (more than 140 g per week for men or 70 g per week for women); (4) known liver disease such as autoimmune liver diseases, viral hepatitis; (5) a lack of required data. Finally, 245 patients were included. A flow chart of study design and patient enrollment is presented in Figure 1. All participants gave written informed consent in accordance with the Declaration of Helsinki. The study was approved by the ethics committee of Changzhou Second People's Hospital Affiliated with Nanjing Medical University (Approval date of Registry and the Registration No. of the study/trial: 1 July 2020, MR-32-21-013406).

Data Collection and Measurements

The patients' general clinical data such as sex, age, height, weight, systolic blood pressure, diastolic blood pressure, and smoking history were collected through electronic medical record review. After the patients fasted for at least 8 h, blood samples were obtained to assess liver biochemistry, lipids, insulin, C-peptide, glucose, and other biochemical indicators.

We then calculated the following index: (1) BMI = weight (in kg)/height^2 (in m^2). The BMI cutoff value of 25 kg/m^2 was used to define the non-obese Asian population;[12] (2) There were two indexes reflecting insulin resistance. The homeostatic model assessment of insulin resistance (HOMA-IR) = fasting insulin (FINS, μU/mL) × fasting blood glucose (FBG, mmol/L)/22.5. Triglyceride-glucose (TyG) index = Ln[TG (mg/dL) × FBG (mg/dL)/2];[13] (3) FIB-4 and APRI were both effective indicators for evaluating liver fibrosis. FIB-4= age (year) ×AST (U/L)/(Platelet count [10^9/L]×(ALT [U/L])$^{1/2}$). APRI = (AST [IU/L])/(AST upper limit of normal [IU/L])/(Platelet count [10^9/L]) ×100.[14,15] In our laboratory, the upper limit of normal of AST was 40 IU/L; (4) There were two indexes reflecting the severity of atherosclerosis.

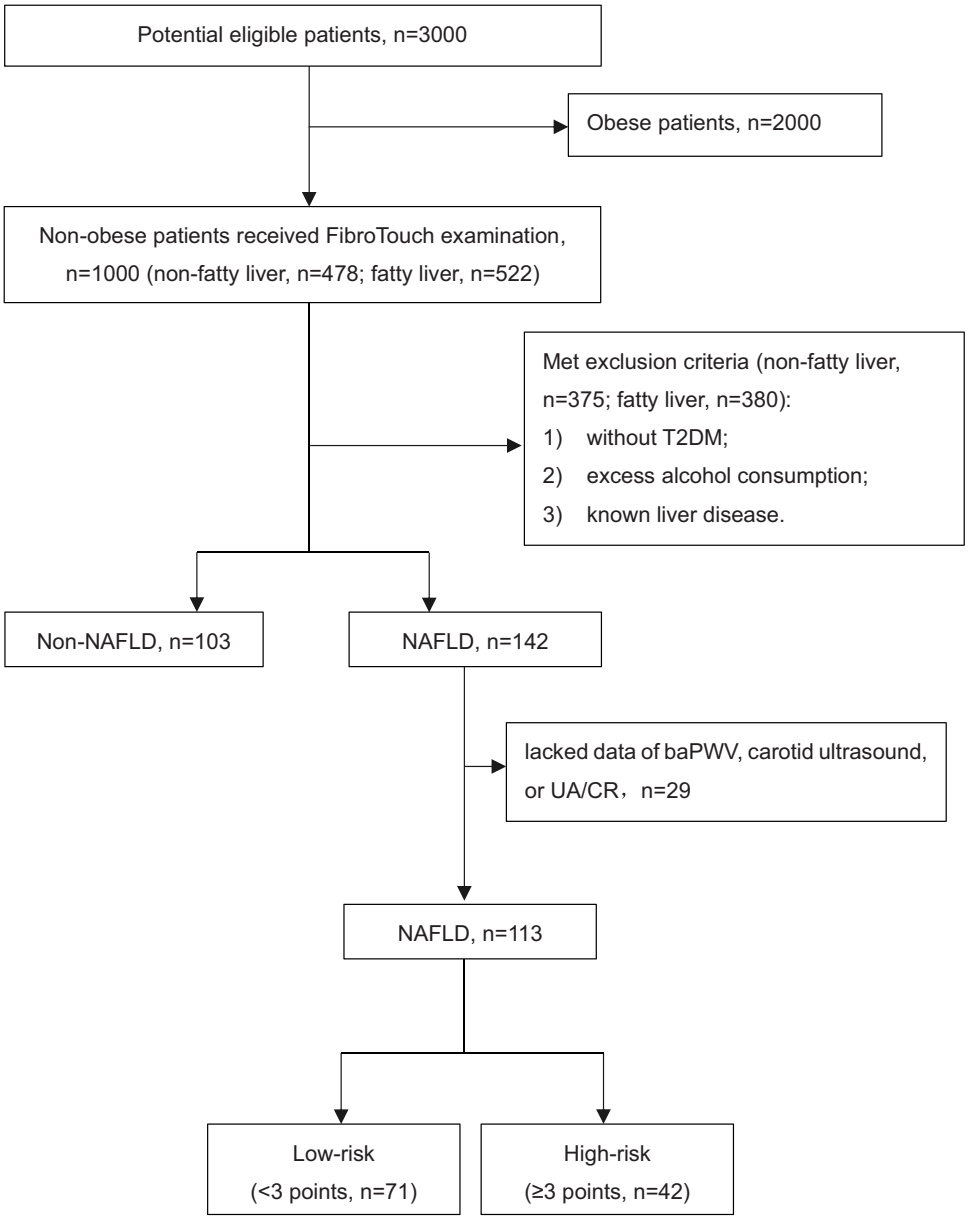

Figure 1 Flow chart of study design and patient enrollment.

Atherosclerosis index (AI)= [TC(mmol/L)-HDL-C(mmol/L)]/HDL-C(mmol/L).[16] Atherogenic index of plasma (AIP)= log[TG(mmol/L)/HDL-C(mmol/L)].[17]

Diagnosis of NAFLD

Transient elastography FibroTouch (FibroTouch-Pro 3800X, iLivTouch series, Wuxi Hisky Medical Technologies, China) was employed for liver examination by a proficient technician with professional training. Following the instructions, patients assumed a supine position on an empty stomach while raising their right hand behind their head to expand the intercostal space. The detection points were selected as the 7–9 intercostal spaces from the right anterior axillary line to the midaxillary line. The ultrasound attenuation parameter (UAP, dB/m) was measured based on the principle that ultrasonic waves propagating in liver tissue are significantly attenuated by lipid droplets in hepatocytes, thereby reflecting the extent of liver steatosis. Additionally, liver stiffness measurement (LSM, kPa) was conducted to evaluate the degree of liver fibrosis. A total of 10 consecutive effective tests were performed, and the median value was considered as the final result (results were

deemed invalid if either ratio of interquartile range to median >30% or operation success rate <60%). NAFLD diagnosis criteria included UAP≥240dB/m according to manufacturer's recommendations.[18]

Assessment of Visceral and Subcutaneous Fat Area

The same trained technicians utilized a visceral fat measuring device (OMRON, HDS-2000, China) according to provided instructions for measuring both visceral and subcutaneous fat areas. This device employs a bioelectrical impedance method that accurately calculates these areas without any radiation risk.

Assessment of Atherosclerosis

After a 15-min rest, the examiner utilized an atherosclerosis detection device (OMRON, BP-203RPE III, China) to simultaneously measure the left and right brachial-ankle pulse wave velocity (baPWV, cm/s) based on transmission time and distance. This device recorded the waveforms of the brachial and anterior tibial arteries. The maximum value from bilateral measurements was considered as the final result. A baPWV≥1400 cm/s indicated presence of arteriosclerosis.[19] A carotid artery B ultrasound examination was conducted by a specialist sonographer to assess carotid intima-media thickness (CIMT). CIMT > 1.0mm was classified as thickened, while carotid plaque was defined as CIMT > 1.5mm or at least 0.5mm greater than surrounding normal CIMT or at least 50% greater than surrounding normal CIMT.[20]

Assessment of Cardiovascular Disease Risk

Studies have demonstrated that baPWV is a robust predictor of cardiovascular events and all-cause mortality.[21] The American Diabetes Association (ADA) has highlighted proteinuria as a risk factor for cardiovascular disease in diabetic patients.[22] A large study discovered that individuals with type 2 diabetes and albuminuria faced a 2.38 and 2.37-fold increased risk of death and major cardiovascular events, respectively, in comparison to those without albuminuria.[23] As urinary microalbumin creatinine ratio (UA/CR) increases, the risk of cardiovascular-related adverse events increases.[24] Carotid intima-media thickening and carotid plaque are considered reliable indicators for assessing cardiovascular disease risk.[20] Therefore, this study combined results from baPWV, carotid ultrasound, and UA/CR to establish scoring criteria for evaluating cardiovascular disease risk in the study population: (1) baPWV: <1400 cm/s, 0 points; ≥1400 cm/s, 1 point; (2) carotid ultrasound: no carotid intima-media thickening and carotid plaque, 0 points; carotid artery thickening 1 point; carotid plaque formation, 2 points; (3) UA/CR: <30 μg/mg, 0 points; 30 μg/mg to 300 μg/mg, 1 point; ≥300 μg/mg, 2 points. In this study, maximum total score was set at five points with scores ≥3 defining high-risk individuals for cardiovascular disease.

Statistical Analysis

The data were analyzed using IBM SPSS Statistics version 26.0 (Chicago, IL, USA). Normally distributed results were expressed as mean ± standard deviation (SD), and group comparisons were performed using unpaired Student's *t*-tests or one-way analysis of variance (ANOVA). Non-normally distributed values were presented as median with interquartile ranges (IQR), and group comparisons were conducted using the Mann–Whitney *U*-test or Kruskal–Wallis *H*-test. Categorical variables were described as frequency and percentage (%), and a chi-square test was employed for group comparisons. Univariate logistic regression analysis was initially used to investigate potential predictors for high-risk population of cardiovascular disease, followed by multivariate analysis to identify independent predictors and their significance. The receiver operating characteristic (ROC) curve analysis was used to evaluate the predictive value of the indicators screened by logistic regression analysis. All tests were two-sided, with a P-value < 0.05 considered statistically significant.

Results

Comparison of Clinical Characteristics Between Non-Obese T2DM Patients with and without NAFLD

A total of 245 non-obese T2DM patients participated in this study, including 103 (42.0%) patients without NAFLD and 142 (58.0%) patients with NAFLD. There was no significant difference in age and gender between the two groups.

Compared to the non-NAFLD group, the NAFLD group exhibited higher BMI, waist circumference (WC), hip circumference (HC), waist–hip ratio (WHR), visceral fat area (VFA) and subcutaneous fat area (SFA) ($P < 0.01$). Fasting C-peptide (FC), fasting insulin (FINS), postprandial insulin (PINS), homeostatic model assessment of insulin resistance (HOMA-IR), and TyG index showed higher values in the NAFLD group ($P < 0.05$). Interestingly, glycosylated hemoglobin (HbA1c) levels were lower in the NAFLD group. However, there was no significant difference in fasting and postprandial blood glucose levels and diabetes duration between the two groups. The serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), γ-glutamyl transpeptidase (γ-GT), uric acid (UA), triglyceride (TG), and total cholesterol (TC) in NAFLD group were significantly higher than those in control group ($P < 0.01$ or $P < 0.05$), but there was no significant difference in high-density lipoprotein-cholesterol (HDL-C) and low-density lipoprotein-cholesterol (LDL-C) between the two groups. The UAP and APRI values were significantly higher in the NAFLD group compared to the non-NAFLD group ($P < 0.01$), but there was no significant difference in LSM and FIB-4 values between the two groups. AIP and AI were also significantly higher in the NAFLD group ($P < 0.01$), but the proportions of carotid intima-media thickening, carotid plaque and the history of smoking were similar between the two groups. The proportion of patients with a history of hypertension was higher in the NAFLD group ($P < 0.05$) (Table 1).

Table 1 Demographic and Clinical Characteristics of Non-Obese T2DM Patients by the Presence of NAFLD

Characteristic	Non-NAFLD (n=103)	NAFLD (n=142)	P-value
Age (year)	52(46,58)	54(47,58)	0.393
Male, n (%)	65(63.1)	75(52.8)	0.108
BMI (kg/m^2)	21.98±1.57	22.85±1.36	<0.001
WC (cm)	80.45±5.36	83.83±5.12	<0.001
HC (cm)	91.00(87.50,93.00)	91.80(89.00,94.00)	0.005
WHR Men Women	0.89±0.05 0.90±0.05 0.88±0.05	0.91±0.05 0.92±0.04 0.90±0.05	0.003 0.015 0.023
VFA (cm^2)	64.47±23.75	77.37±20.12	<0.001
SFA (cm^2)	131.91±27.34	153.58±32.04	<0.001
SBP (mmHg)	119±16	122±17	0.224
DBP (mmHg)	73±10	73±11	0.938
History of HT, n (%)	30(29.1)	61(43.0)	0.027
Smoke, n (%)	30(29.1)	29(20.4)	0.116
Course of T2DM (month)	88(28,126)	62(8,126)	0.098
FBG (mmol/L)	6.84(5.40,8.73)	6.99(5.83,9.17)	0.409
PBG (mmol/L)	15.26±4.81	15.00±4.33	0.713
FINS (pmol/L)	23.210(8.970,33.380)	33.825(23.203,47.415)	<0.001
PINS (pmol/L)	94.770(46.405,199.775)	140.400(78.310,274.200)	0.047
HOMA-IR	1.07(0.43,1.82)	1.66(1.10,2.87)	<0.001

(*Continued*)

Table 1 (Continued).

Characteristic	Non-NAFLD (n=103)	NAFLD (n=142)	P-value
TyG index	8.76±0.66	9.09±0.70	0.001
FC (pmol/L)	352.000(194.900,536.000)	500.450(321.800,714.725)	<0.001
PC (pmol/L)	918.050(503.800,1794.250)	1081.500(639.625,1702.750)	0.375
HbA1c (%)	9.7(7.4,11.5)	8.7(7.1,10.5)	0.023
ALT (U/L)	12.3(9.0,19.0)	17.0(12.5,25.7)	<0.001
AST (U/L)	14.0(12.2,17.3)	16.5(14.0,20.9)	<0.001
ALP (U/L)	67.0(57.0,81.0)	73.0(58.5,88.5)	0.063
γ-GT (U/L)	16.0(11.0,21.0)	19.0(14.0,28.0)	<0.001
BUN (mmol/L)	5.5(4.4,6.7)	5.2(4.4,6.5)	0.360
Cr (umol/L)	63.0(55.0,74.5)	61.0(51.0,73.8)	0.337
UA (umol/L)	270.3±72.7	292.8±91.4	0.045
TG (mmol/L)	1.17(0.82,1.53)	1.46(1.13,2.17)	<0.001
TC (mmol/L)	4.07±0.77	4.32±0.99	0.035
HDL-C (mmol/L)	1.07(0.88,1.23)	1.03(0.89,1.24)	0.715
LDL-C (mmol/L)	2.58(2.20,3.10)	2.48(1.99,2.96)	0.157
UA/Cr	10.75(6.85,28.52)	9.85(6.13,18.95)	0.295
UAP (dB/m)	221.097(214.246,230.140)	263.948(254.184,278.264)	<0.001
LSM (kPa)	5.484±1.289	5.490±1.035	0.970
FIB-4	0.968(0.754,1.286)	0.931(0.746,1.364)	0.930
APRI	0.18(0.13,0.22)	0.19(0.15,0.27)	0.010
CIMT > 1.0 mm, n (%)	28(34.1)	40(34.8)	0.926
Carotid plaque, n (%)	49(57.6)	54(46.6)	0.120
AIP	0.029±0.251	0.141±0.224	0.001
AI	2.760±0.787	3.044±0.989	0.021

Notes: Continuous values consistent with normal distribution are shown as mean ± SD, while non-normally distributed values are presented as median with IQR. Categorical values are shown as frequency and percentage (%).

Abbreviations: BMI, body mass index; WC, waist circumference; HC, hip circumference; WHR, waist-to-hip ratio; VFA, visceral fat area; SFA, subcutaneous fat area; SBP, systolic blood pressure; DBP, diastolic blood pressure; HT, hypertension; T2DM, type 2 diabetes; FBG, fasting blood glucose; PBG, postprandial blood glucose; FINS, Fasting insulin; PINS, postprandial insulin; HOMA-IR, homeostasis model assessment of insulin resistance; TyG index, triglyceride-glucose index; FC, fasting C-peptide; PC, postprandial C-peptide; HbA1c, glycosylated hemoglobin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; γ-GT, γ-glutamyl transpeptidase; BUN, blood urea nitrogen; Cr, creatinine; UA, uric acid; TG, triglyceride; TC, total cholesterol; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; UACr, urinary microalbumin creatinine ratio; UAP, ultrasound attenuation parameter; LSM, liver stiffness measurement; APRI, AST to platelet ratio index; CIMT, carotid intima-media thickness; AIP, atherogenic index of plasma; AI, atherosclerosis index.

Clinical Characteristics Among Non-Obese T2DM Patients with NAFLD Grouped by UAP

In order to observe the changes in various clinical indicators as the degree of fatty liver increased, we divided the non-obese T2DM patients with NAFLD into three groups based on UAP tertiles (T1: n=47, UAP<256.97; T2: n=48, 256.97≤UAP<272.44; T3: n=48, UAP≥272.44). The median with IQR of UAP in the three groups was 50.801

(242.931, 254.195), 263.947 (261.882, 267.097) and 282.375 (278.230, 290.388), respectively. BMI, WC, WHR, VFA, SFA, postprandial blood glucose (PBG), FINS, PINS, FC, PC, HOMA-IR, TyG index, γ-GT, TG, UAP, LSM, AIP and AI in T3 group were higher than those in T1 group ($P < 0.05$) (Table 2).

Table 2 Demographic and Clinical Characteristics of Non-Obese T2DM Patients with NAFLD by UAP

Characteristic	T1(n=47) UAP<256.97	T2(n=48) 256.97≤UAP<272.44	T3(n=47) UAP>272.44	P-value
Age (year)	55(46,61)	52(47,58)	53(47,58)	0.708
Male, n(%)	27(57.4)	27(56.3)	21(44.7)	0.391
BMI ($kg/m^{2)}$	22.90(21.70,23.60)	22.80(21.70,23.82)	23.30(22.70,23.90)[ab]	0.015
WC (cm)	82.07±6.35	84.44±4.20[a]	85.17±3.82[a]	0.01
HC (cm)	91.00(87.70,94.00)	91.50(89.00,94.00)	92.00(90.00,94.00)	0.802
WHR	0.89±0.05	0.92±0.03[a]	0.91±0.04[a]	<0.001
VF (cm^2)	67.84±21.74	82.51±19.92[a]	81.77±15.02[a]	0.001
SF (cm^2)	143.18±35.88	155.50±30.88	161.61±26.94[a]	0.030
SBP (mmHg)	121±17	121±18	123±14	0.730
DBP (mmHg)	72±11	73±12	74±9	0.548
History of HT, n(%)	20(42.6)	18(37.5)	23(48.9)	0.302
Smoke, n(%)	9(19.1)	12(25)	8(17)	0.606
Course of T2DM (month)	79(13,154)	50(4,126)	62(13,122)	0.732
FBG (mmol/L)	6.94(5.76,9.38)	6.97(5.84,9.75)	7.15(5.91,8.78)	0.976
PBG (mmol/L)	13.22±4.41	15.40±4.70[a]	16.10±3.46[a]	0.020
FINS (pmol/L)	26.590(11.970,41.585)	32.990(20.570,46.283)	42.660(29.905,68.515)[a]	0.020
PINS (pmol/L)	99.660(49.765,177.350)	132.100(60.795,266.750)	170.000(133.000,402.000)[a]	0.007
HOMA-IR	1.40(0.73,2.23)	1.62(0.99,3.00)	1.99(1.33,3.97)[a]	0.048
TyG index	8.83±0.62	9.17±0.73[a]	9.22±0.70[a]	0.022
FC (pmol/L)	379.902±234.386	553.111±286.514[a]	654.956±282.991[a]	<0.001
PC (pmol/L)	854.391±612.068	1342.126±830.481[a]	1532.263±730.221[a]	<0.001
HbAlc (%)	8.9(7.5,10.4)	8.7(7.0,10.6)	8.1(7.0,10.6)	0.829
ALT (U/L)	16.0(11.0,22.0)	17.0(11.2,22.4)	19.0(13.7,28.3)	0.154
AST (U/L)	16.1(12.2,20.0)	16.0(13.4,19.1)	17.1(14.2,21.4)	0.392
ALP (U/L)	77.0(64.0,100.0)	75.0(55.0,87.0)	70.0(58.0,83.0)	0.452
γ-GT (U/L)	16.0(12.0,24.0)	21.5(14.0,28.8)	22.0(17.0,31.0)[a]	0.012
BUN (mmol/L)	5.7(4.8,6.8)	5.1(4.3,6.3)	5.0(4.3,6.1)	0.138
Cr (umol/L)	65.0(56.0,75.0)	59.0(51.0,73.6)	60.0(49.0,75.0)	0.430
UA (umol/L)	292.9±73.4	280.1±94.7	305.3±103.5	0.419
TG (mmol/L)	1.37(0.93,1.89)	1.40(1.16,2.41)	1.63(1.21,2.33)[a]	0.031

(*Continued*)

Table 2 (Continued).

Characteristic	T1(n=47) UAP<256.97	T2(n=48) 256.97≤UAP<272.44	T3(n=47) UAP>272.44	P-value
TC (mmol/L)	4.35±1.06	4.40±0.99	4.21±0.94	0.648
HDL-C (mmol/L)	1.05(0.93,1.19)	1.14(0.91,1.26)	0.97(0.87,1.20)	0.272
LDL-C (mmol/L)	2.64±0.93	2.59±0.88	2.50±0.68	0.650
UA/Cr	8.90(5.80,22.55)	10.55(6.30,18.95)	9.85(6.55,17.65)	0.893
Proteinuria, n(%)	5(10.6)	3(6.3)	4(8.5)	0.744
UAP (dB/m)	250.801(242.931,254.195)	263.947(261.882,267.097)[a]	282.375(278.230,290.388)[ab]	<0.001
LSM (kPa)	5.272±0.921	5.322±0.930	5.915±1.159[ab]	0.012
FIB-4	0.937(0.755,1.419)	0.967(0.807,1.397)	0.885(0.646,1.216)	0.224
APRI	0.18(0.15,0.28)	0.20(0.15,0.25)	0.19(0.15,0.27)	0.990
CIMT > 1.0 mm, n (%)	10(27.8)	17(39.5)	13(36.1)	0.308
Carotid plaque, n (%)	20(55.6)	22(50.0)	12(33.3)	0.092
AIP	0.084±0.216	0.123±0.245	0.225±0.190[a]	0.019
AI	2.879±1.039	2.869±0.944	3.379±0.912[ab]	0.027

Notes: [a]*P*<0. 05 compared with Tertile 1, [b]*P*<0. 05 compared with Tertile 2.

Univariate and Multivariate Logistic Regression Analyses of Identification Factors of Cardiovascular Disease in Non-Obese T2DM Patients with NAFLD

As illustrated in Figure 1, a total of 142 non-obese T2DM patients with NAFLD were enrolled in our study, with 29 of them being excluded due to the lack of data (baPWV, carotid ultrasound or UA/CR) required for cardiovascular risk assessment in this study. Finally, 113 non-obese T2DM patients with NAFLD were included for further cardiovascular risk assessment. According to the cardiovascular risk assessment criteria of this study, 42 patients (37.2%) were at high risk and 71 patients (62.8%) were at low risk (Figure 2). Univariate logistic regression analysis showed that age, systolic blood pressure, duration of diabetes, LDL-C, FIB-4, AIP and history of hypertension were independent risk factors for cardiovascular disease in non-obese T2DM patients with NAFLD, all of which were identified to create the multivariate logistic regression model. The Hosmer–Lemeshow goodness-of-fit test indicated that the predictive model was well calibrated (χ^2 = 4.678, P = 0.791). Multivariate logistic regression analysis showed that age (OR 1.153; 95% CI 1.016–1.308, P=0.027), systolic blood pressure (OR 1.068; 95% CI 1.017–1.121, P=0.008), LDL-C (OR 10.191; 95% CI 2.539–40.916, P=0.001) and AIP (OR 46.211; 95% CI 1.148–1860.121, P=0.042) were still independent risk factors for cardiovascular disease in non-obese T2DM patients with NAFLD (Table 3).

Predictive Value of the Identified Factors of Cardiovascular Disease in Non-Obese T2DM Patients with NAFLD

We further explore the application of the factors identified from the logistic model in an ROC curve analysis. LDL-C, which had the highest AUROC among these indicators, had the best discrimination capacity (AUROC: 0.738, 95% CI: 0.622–0.853, cutoff value: 2.97mmol/L; P<0.001), whereas AIP had the worst performance (AUROC: 0.642, 95% CI: 0.520–0.764, cutoff value: 0.002; P=0.033). Age (AUROC: 0.705, 95% CI: 0.592–0.818, cutoff value: 50 years; P=0.002) and systolic blood pressure (SBP) (AUROC: 0.688, 95% CI: 0.569–0.807, cutoff value: 121mmHg; P=0.005) had moderate predictive efficacy. Furthermore, the combination of LDL-C, age, SBP, and AIP had a significant AUROC of 0.895 (P<0.001) (Figure 3).

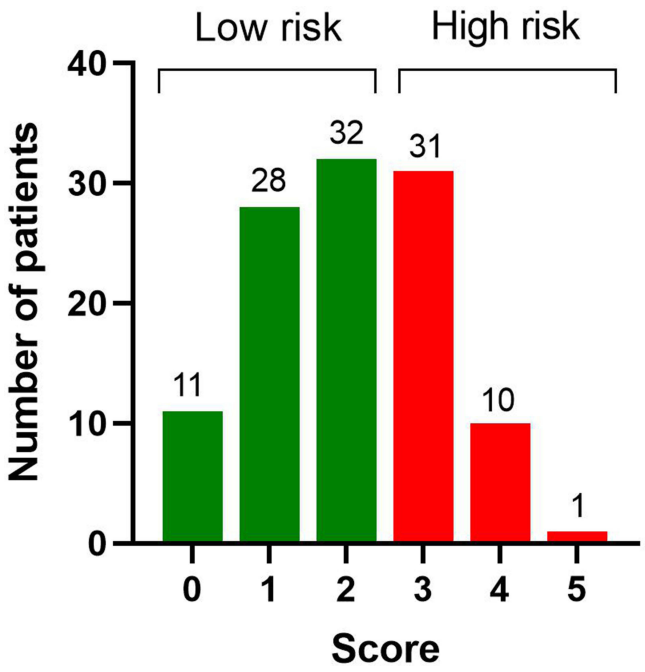

Figure 2 Risk scores of cardiovascular disease in non-obese T2DM patients with NAFLD.

Discussion

In this study, we found that in the non-obese T2DM population, BMI, waist circumference, hip circumference, waist-to-hip ratio, visceral fat, and subcutaneous fat were higher in patients with NAFLD compared to non-NAFLD patients. BMI, waist circumference, waist-to-hip ratio, visceral fat, and subcutaneous fat were also higher in patients with more

Table 3 Univariate and Multivariate Logistic Regression Analyses of Risk Factors of Cardiovascular Disease in Non-Obese T2DM Patients with NAFLD

Exposure	Univariate			Multivariate		
	OR	(95% CI)	P-value	OR	(95% CI)	P-value
Age (year)	1.114	(1.051–1.180)	<0.001	1.153	(1.016–1.308)	0.027
BMI (kg/m^2)	0.996	(0.735–1.349)	0.978			
WC (cm)	0.993	(0.923–1.068)	0.855			
HC (cm)	0.934	(0.851–1.024)	0.147			
WHR	43.269	(0.058–32,211.878)	0.264			
VF (cm^2)	1.006	(0.989–1.023)	0.493			
SF (cm^2)	1.001	(0.989–1.013)	0.913			
SBP (mmHg)	1.033	(1.008–1.058)	0.009	1.068	(1.017–1.121)	0.008
DBP (mmHg)	1.007	(0.972–1.043)	0.713			
Course of T2DM (month)	1.007	(1.002–1.012)	0.011	1.002	(0.993–1.011)	0.625
FBG (mmol/L)	0.892	(0.779–1.021)	0.098			

(*Continued*)

Table 3 (Continued).

Exposure	Univariate			Multivariate		
	OR	(95% CI)	P-value	OR	(95% CI)	P-value
PBG (mmol/L)	1.002	(0.913–1.101)	0.958			
FINS (pmol/L)	0.998	(0.985–1.012)	0.789			
PINS (pmol/L)	0.999	(0.996–1.003)	0.669			
HOMA-IR	0.952	(0.778–1.166)	0.637			
TyG index	0.775	(0.431–1.392)	0.393			
FC (pmol/L)	1.001	(0.999–1.002)	0.442			
PC (pmol/L)	1.000	(1.000–1.000)	0.952			
HbAlc (%)	0.918	(0.768–1.097)	0.345			
Hb (g/L)	0.983	(0.957–1.009)	0.197			
Platelet count (10^9/L)	1.000	(0.994–1.007)	0.884			
ALT (U/L)	1.004	(0.991–1.017)	0.568			
AST (U/L)	1.013	(0.989–1.037)	0.303			
ALP (U/L)	1.003	(0.996–1.010)	0.355			
γ-GT (U/L)	1.005	(0.997–1.013)	0.234			
BUN (mmol/L)	1.080	(0.851–1.371)	0.527			
Cr (umol/L)	1.019	(0.997–1.041)	0.091			
UA (umol/L)	1.003	(0.999–1.007)	0.196			
TG (mmol/L)	0.780	(0.550–1.106)	0.164			
TC (mmol/L)	1.149	(0.778–1.696)	0.484			
HDL-C (mmol/L)	2.107	(0.481–9.238)	0.323			
LDL-C (mmol/L)	4.761	(2.109–10.746)	<0.001	10.191	(2.539–40.916)	0.001
UAP (dB/m)	0.989	(0.965–1.014)	0.376			
LSM (kPa)	0.786	(0.613–1.007)	0.057			
FIB-4	2.922	(1.149–7.434)	0.024	1.603	(0.187–13.705)	0.667
APRI	2.768	(0.380–20.152)	0.315			
AIP	20.094	(2.129–189.619)	0.009	46.211	(1.148–1860.121)	0.042
AI	0.972	(0.637–1.484)	0.896			
Male	0.746	(0.347–1.603)	0.453			
History of HT	3.312	(1.492–7.350)	0.003	2.937	(0.723–11.930)	0.132
Smoke	0.747	(0.291–1.920)	0.545			

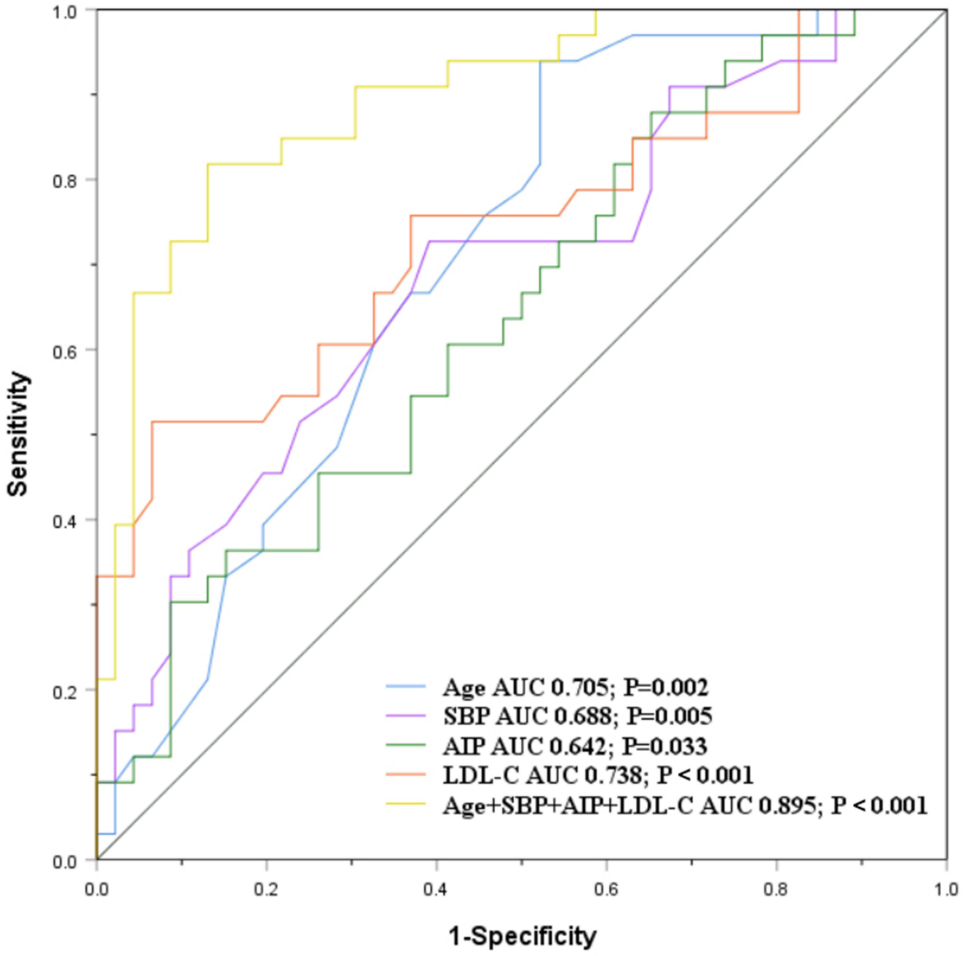

Figure 3 Receiver operator characteristic (ROC) curve that predicts cardiovascular disease for non-obese T2DM patients with NAFLD.

severe fatty liver than in those with less severe fatty liver. Correspondingly, a prospective study in Korea found that the prevalence of NAFLD increased with weight gain in the normal BMI range in the non-obese population.[25] Another prospective study reported remission of NAFLD after 3–5% weight loss in non-obese NAFLD patients.[26] This suggests that weight gain, especially increase in adipose tissue, plays an important role in the development of non-obese NAFLD.

We found that the HOMA-IR and TyG index related to insulin resistance were higher in NAFLD patients than in non-NAFLD patients, and insulin resistance was more pronounced as the degree of fatty liver increased. Adipose tissue has a special role in insulin resistance (IR). Insulin can promote lipid synthesis and inhibit its degradation. When insulin resistance occurs, not only blood glucose is elevated, but also lipids, and elevated circulating free fatty acids from adipocytes can further exacerbate insulin resistance by inhibiting glucose uptake, glycogen synthesis, and glucose oxidation, as well as by increasing hepatic glucose output.[27] IR is not only related to obese NAFLD, but also participates in the pathogenesis of non-obese NAFLD.[28] A study found that IR was an independent predictor of NAFLD, independent of BMI.[29] Hyperinsulinemia directly promotes NAFLD by promoting the expression of key enzymes required for fatty acid synthesis in the liver, increasing the content of de novo fatty acid synthesis in the liver, and indirectly promotes the occurrence of NAFLD by promoting lipolysis of peripheral fat and thereby increasing fatty acid inflow into the liver.[30] An animal study found that adipose tissue-specific insulin receptor gene knockout mice developed more severe NAFLD, with histologic manifestations of progressively worsening hepatocyte ballooning degeneration.[31] Clinical studies have shown that the condition of NAFLD patients could be significantly improved after treatment with insulin sensitizers.[32] A study in a non-obese, non-diabetic population showed that hepatic triglyceride content contributed more to the development of insulin resistance compared to waist circumference and BMI.[33] Hepatic steatosis activates the NF-κB signaling pathway,

induces hepatic inflammatory responses, and upregulates the expression of pro-inflammatory factors, which mediate hepatic and systemic insulin resistance and are involved in the pathogenesis of cardiovascular disease.[34–36] Therefore, insulin resistance and NAFLD may be causally related to each other.

A cross-sectional study found that atherogenic dyslipidemia was independently associated with NAFLD after adjusting for IR and obesity.[37] One study reported comparable lipid profiles in obese and non-obese NAFLD.[38] Therefore, NAFLD itself may cause dyslipidemia, thereby increasing the risk of cardiovascular disease in patients with NAFLD. It has been suggested that NAFLD-induced atherosclerotic dyslipidemia is driven by increased hepatic lipid synthesis.[39] In the present study, we found that the atherosclerosis-related indices AI and AIP were higher in patients with NAFLD compared with those without NAFLD, and both of these indices were higher as the severity of fatty liver increased, suggesting an association between hepatic triglyceride content and atherosclerosis. In addition, patients with NAFLD had a higher prevalence of hypertension, which is consistent with the results of a previous study.[30] In conclusion, NAFLD may be a risk factor for cardiovascular disease due to its concomitant inflammatory response, insulin resistance, dyslipidemia, and hypertension.

Age, systolic blood pressure, high LDL-C and low HDL-C are recognized as risk factors for cardiovascular disease in the general population.[40] AIP, an emerging index calculated by combining TG and LDL-C, is an independent risk factor for non-obese NAFLD.[17] AIP is closely associated with plasma lipoprotein particle size and can be used for risk assessment of cardiovascular disease.[41,42]

At present, there are few studies on the risk of cardiovascular disease in non-obese T2DM patients with NAFLD, and there is a lack of reference for the prevention and treatment of cardiovascular disease in this population. The strength of this study is that in light of previous cardiovascular risk studies, this research employed baPWV, UA/CR, and carotid ultrasound results to develop a cardiovascular risk assessment system, categorizing individuals with scores ≥3 as high-risk groups. Given that these indicators are easily accessible in clinical practice, it offers a straightforward and convenient method for identifying high-risk patients in this population. Furthermore, this study demonstrated that age, systolic blood pressure, AIP, and LDL-C were strong predictors of CVD risk in non-obese T2DM patients with NAFLD. For this population, clinical attention should be paid to the management of serum lipids and blood pressure to prevent cardiovascular disease.

The primary limitation of this research is its inability to assess the efficacy of existing scoring system. Consequently, larger-scale studies are required to evaluate the effectiveness of this scoring system in identifying individuals with a high risk of cardiovascular diseases in the future. Given that this study is a single-center retrospective study with an average sample size, the results need to be further confirmed by clinical prospective studies, so as to identify, manage and treat non-obese T2DM patients with NAFLD at high risk of cardiovascular disease more effectively.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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