ORIGINAL PAPER

The Mediation Effects of Type 2 Diabetes Mellitus and Related Biomarkers on the Association of Metabolic Dysfunction-associated Steatotic Liver Disease and Fibrosis

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ABSTRACT

Background & Aims: Both metabolic dysfunction-associated steatotic liver disease (MASLD) and fibrosis have been associated with type 2 diabetes mellitus (T2DM), but the roles of T2DM and related biomarkers in the association between MASLD and fibrosis are yet to be fully elucidated. This study aimed at assessing whether the association between MASLD and fibrosis is mediated by T2DM.

Methods: A total of 6,060 participants from NHANES 2017-2020 were enrolled in the cross-sectional analyses. Pairwise associations among MASLD, fibrosis, T2DM, and T2DM-related biomarkers [plasma fasting glucose, hemoglobin A1c (HbA1c), serum insulin, and homeostatic model assessment for insulin resistance (HOMA-IR)] were examined, and then the extent to which MASLD progresses to fibrosis through T2DM and the biomarkers was assessed.

Results: We found a higher risk of T2DM and higher levels of T2DM-related biomarkers were associated with MASLD. Moreover, T2DM and higher levels of T2DM-related biomarkers were positively associated with fibrosis risk. T2DM, plasma fasting glucose, HbA1c, serum insulin, and HOMA-IR mediated 10.1%, 9.99%, 10.5%, 5.98%, and 7.28% of the association between MASLD and fibrosis, respectively. In addition, the mediation effect of T2DM varied in different groups of age, body mass index, and antidiabetic medication.

Conclusions: T2DM and T2DM-related biomarkers partly mediated the association between MASLD and fibrosis.

Key words: metabolic dysfunction- associated steatotic liver disease – fibrosis – type 2 diabetes – mediation analysis.

Abbreviations: BMI: body mass index; CAP: controlled attenuation parameter; HbA1c: hemoglobin A1c; HOMA-IR: homeostatic model assessment for insulin resistance; IQR: interquartile range; IR: insulin resistance; LSM: liver stiffness measurement; MASLD: metabolic dysfunction-associated steatotic liver disease; NAFLD: non-alcoholic fatty liver disease; NHANES: National Health and Nutrition Examination Survey; T2DM: type 2 diabetes mellitus.

INTRODUCTION

Recently, to emphasize the critical role of metabolic dysfunction in the disease progression and avoiding stigmatization, an expert panel has renamed non-alcoholic fatty liver disease (NAFLD) to metabolic dysfunction-associated steatotic liver disease (MASLD) [1]. MASLD is currently a prominent contributor to liver-related deaths globally, affecting 32.45% of adults [2]. According to the current investigation, 33.6% of individuals with MASLD had fibrosis progression, which put them at risk of developing end-stage liver disease and hepatocellular carcinoma [3, 4]. Additionally, MASLD displays heterogeneity in terms of the disease progression rate and clinical outcomes, potentially influenced by varying factors associated with its development [5]. However, underlying mechanisms of fibrosis progression in MASLD patients have been rarely reported.

Type 2 diabetes mellitus (T2DM) is a widely encountered metabolic disorder and linked to MASLD and fibrosis [6-8]. It is known that the association of MASLD with T2DM is strong and bidirectional and MASLD is frequently coexisted with T2DM [7]. The global prevalence of MASLD among individuals with T2DM was estimated to be 55.5% [9]. Moreover, the diagnosis of MASLD was linked to an

approximately doubled risk of T2DM [10]. Meanwhile, T2DM is related to fibrosis development. The prior evidence suggested that there was a markedly elevated cumulative incidence of fibrosis progression in patients with T2DM compared with those without T2DM in the long term [11]. Considering the associations among MASLD, T2DM and fibrosis, it is plausible that T2DM plays an important role in the connection between MASLD and fibrosis. However, no observational studies have yet utilized analytical techniques to determine the degree to which T2DM and its related biomarkers contribute to the increased risk of fibrosis in those with MASLD. Understanding the potential mechanisms and the mediation effects of T2DM and associated biomarkers in the nexus between fibrosis and MASLD are crucial for reducing fibrosis risk in MASLD patients. Focusing on T2DM prevention or treatment might provide a viable strategy to mitigate the progression from MASLD to fibrosis.

In this study, we posited that the association between MASLD and fibrosis might be partially mediated by T2DM. To test this hypothesis, we used the dataset sourced from the National Health and Nutrition Examination Survey (NHANES) spanning 2017-2020 to verify this relationship. Our research sought to decompose the total effect of MASLD on the risk of fibrosis into the indirect effect (the proportion mediated by T2DM and related biomarkers) and the direct effect (the proportion independent of these mediators) to assess the mediation role of T2DM.

METHODS

Study Population

Participants in our study were from the National Health and Nutrition Examination Survey (NHANES) administered by the National Center for Health Statistics (NCHS). NHANES was a biennial cross-sectional survey that was conducted on a sample that represented the diverse population of the United States. The data collection process involved in-person interviews, physical examinations, and laboratory tests. The survey received ethical approval from the NCHS Ethics Review Board, and all participants provided written informed consent. We utilized data from the 2017-2020 cycle of NHANES, which was publicly accessible.

There were 15,560 participants enrolled in the 2017-2020 cycle. Participants were excluded due to the following reasons: (1) age <18 years old (n=5,867); (2) ineligibility for elastography examination (n=728); (3) the elastography examination status was partial (n=616), ineligible (n=348) or not done (n=233); (4) excessive alcohol consumption (women ≥2 or men ≥3 standard drinks/day) (n=1,317); (5) positivity for hepatitis B surface antigen (n=37), hepatitis C RNA (n=60) or hepatitis C antibody (n=68); (6) elevated transferrin saturation (≥50%) (n=216); (7) missing diabetes questionnaire data (n=1); (8) presence of the type 1 diabetes (diabetes diagnosis prior to the age of 30, initiation of insulin treatment within 2 years of diagnosis, and current use of insulin) (n=9). Finally, our study included 6,060 participants in total. The process of participant inclusion and exclusion in the study is depicted in Fig. 1.

Definitions of NAFLD, MASLD and Liver Fibrosis

The NHANES 2017-2020 employed the controlled attenuation parameter (CAP) to assess liver fat [12]. The CAP assessments were conducted utilizing the FibroScan, whose accuracy in gauging liver steatosis and fibrosis was evaluated [13]. The device automatically computed the median CAP, with the corresponding interquartile range (IQR). CAP values fell within the 100 to 400 dB/m range, where elevated values corresponded to increased levels of hepatic steatosis [13]. In our study, NAFLD was diagnosed as a median CAP value of ≥285 dB/m. MASLD was defined as a median CAP value of ≥285 dB/m with one or more following cardiometabolic risk factors: BMI ≥25 kg/m^2 or waist circumference >80 cm for women and >94 cm for men; fasting glucose ≥5.6 mmol/L, HbA1c ≥ 5.7%, diagnosed type 2 diabetes or treatment for type 2 diabetes; blood pressure >130/85 mmHg, diagnosed hypertension or antihypertensive drug treatment; plasma triglycerides ≥ 150 mg/dL or lipid-lowering treatment; and plasma HDL-cholesterol < 50 mg/dL for women and <40 mg/dL for men or lipid-lowering treatment [1].

Fibrosis was diagnosed by the liver stiffness measurement (LSM), which was also measured by FibroScan. LSM spanned a range of 1.5 kPa to 75 kPa, where higher values were indicative of a greater severity of fibrosis [14]. Fibrosis was diagnosed as having an LSM value of ≥8.0 kPa [15].

T2DM and T2DM-related Biomarkers

Type 2 diabetes mellitus was identified based on individuals' self-reported diagnoses by a physician or healthcare professional [16]. Importantly, the NHANES 2017-2020 questionnaire assessment specifically excluded gestational diabetes. T2DM-related biomarkers included plasma fasting glucose, HbA1c, serum insulin, and HOMA-IR. Plasma fasting glucose was measured using the hexokinase method. HbA1c was determined in whole blood samples through high-performance liquid chromatography (HPLC). Serum insulin levels were evaluated using the AIA-PACK IRI, a two-site immunoenzymometric assay, on the Tosoh AIA System analyzer. A detailed description of the laboratory techniques is available on the NHANES website[17]. The homeostasis model assessment (HOMA) has been proposed as a practical means to estimate insulin sensitivity and pancreatic function and evaluate insulin resistance (IR). HOMA-IR was calculated according to the following formula: HOMA-IR = (fasting plasma insulin [µU/ml] × fasting plasma glucose [mmol/l])/22.5 [18].

Covariates

Based on the directed acyclic graph [19], we made the assumption that a common set of variables may act as potential confounders in the associations between MASLD and fibrosis, MASLD and T2DM, and T2DM and fibrosis (Fig. 2). The measured confounding variables were age, gender, race, and body mass index (BMI) [20-22]. Age (18-44/45-64/≥65), gender (male/female), race (Mexican American/other Hispanic/non-Hispanic White/non-Hispanic Black/non-Hispanic Asian/other race - including multi-racial), BMI (<18.5/18.5-24.9/25-29.9/≥30) were considered as categorical variables.
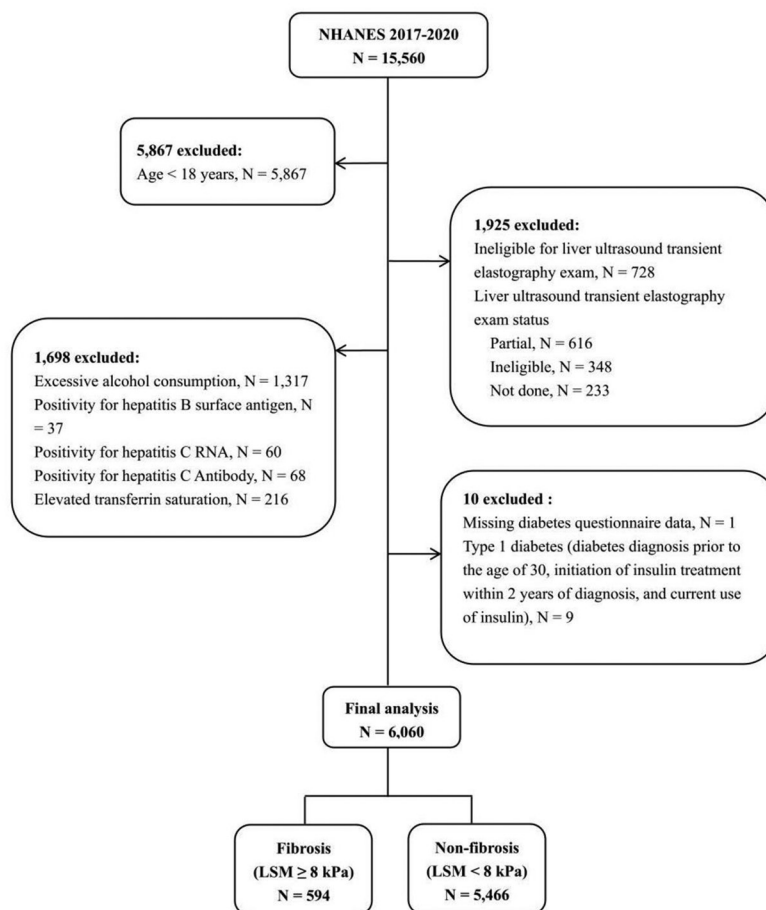
Fig. 1. Flowchart of participants for the study. NHANES: National Health and Nutrition Examination Survey.

In addition, the use of antidiabetic medication was considered as a covariate in the mediating effect of T2DM in our study. If GLP-1 receptor agonists, SGLT-2 inhibitors, and pioglitazone were contained in the participants' prescription history in the past 30 days, they were recorded as receiving antidiabetic medication. The types of antidiabetic medication included in the study and the corresponding drug codes were listed in Supplementary file.
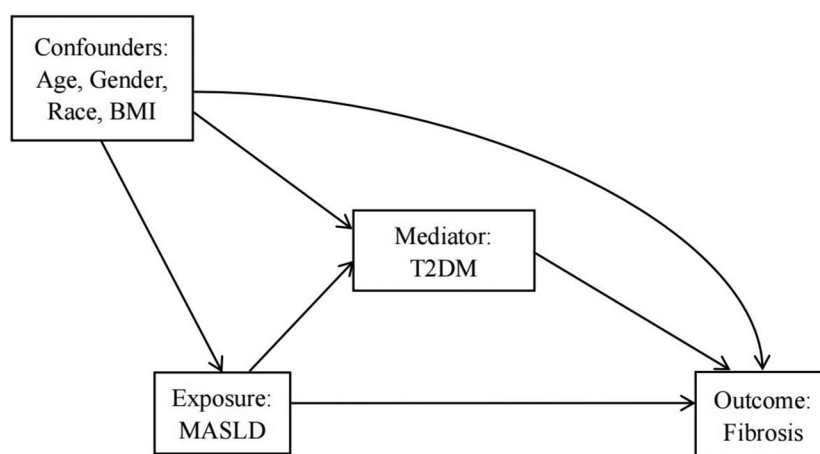
Fig. 2. Hypothesized directed acyclic graph of the relationship among MASLD, fibrosis and T2DM, where "confounders" denote the same set of demographic and examination confounders of the associations between MASLD and fibrosis, MASLD and T2DM, and T2DM and fibrosis. MASLD: metabolic dysfunction-associated steatotic liver disease; T2DM: type 2 diabetes mellitus; BMI: body mass index.

Statistical Analysis

Characteristics of study participants were delineated as follows: continuous variables were depicted using medians and interquartile ranges (IQR), while categorical variables were presented as counts (n) and percentages (%). We employed multivariable logistic regression models to assess the relationships between MASLD and fibrosis, MASLD and T2DM, T2DM and fibrosis, and T2DM-related biomarkers and fibrosis. To gauge the associations between MASLD and T2DM-related biomarkers, we used multivariate linear regression. Two analytical models were formed: (1) The crude model, which was unadjusted; and (2) The adjusted model, which was adjusted in potential confounders such as age, gender, race, and BMI.

To determine the possible mediation roles of T2DM and its associated biomarkers in the nexus between MASLD and fibrosis, we employed the R package "*mediation*" to estimate the indirect effect size ($\beta_{indirect}$), the direct effect size (β_{direct}), the total effect size (β_{total}) and the proportion mediated. Our analysis involved 1000 bootstrap iterations to generate bias-corrected confidence intervals. All mediation analyses considered various covariates, including age, gender, race, and BMI. Recognizing that different MASLD diagnostic criteria might influence mediation analysis outcomes, we executed a sensitivity analysis. For this, we modified the MASLD diagnostic criterion from CAP ≥285 dB/m to CAP ≥274 dB/m, marking a threshold with 90% sensitivity and 60% specificity[23].

All statistical analyses were performed utilizing R software version 4.2.2. (R Foundation for Statistical Computing, Vienna, Austria). Statistical significance was defined as having two-sided p values <0.05.

RESULTS

Of the 6,060 participants in our study, as detailed in Table I, 594 (9.80%) were diagnosed with fibrosis. The presence of fibrosis was notably associated with distinct characteristics. Specifically, those with fibrosis, in comparison to participants

Table I. Characteristics of adults (18+) according to the presence of fibrosis: NHANES 2017–2020

Variables	Total sample (n=6060)	Fibrosis (n=594)	Non-fibrosis (n=5466)	p-value
Age (years)				<0.001
18-44	2268 (37.43)	133 (22.39)	2135 (39.06)	
45-64	2176 (35.91)	246 (41.41)	1930 (35.31)	
≥65	1616 (26.67)	215 (36.20)	1401 (25.63)	
Female, n (%)	3167 (52.26)	251 (42.26)	2916 (53.35)	<0.001
Race/ethnicity, n (%)				0.163
Mexican American	646 (10.66)	66 (11.11)	580 (10.61)	
Other Hispanic	611 (10.08)	61 (10.27)	550 (10.06)	
Non-Hispanic White	2037 (33.61)	211 (35.52)	1826 (33.41)	
Non-Hispanic Black	1659 (27.38)	166 (27.95)	1493 (27.31)	
Non-Hispanic Asian	813 (13.42)	58 (9.76)	755 (13.81)	
Other Race - Including Multi-Racial	294 (4.85)	32 (5.39)	262 (4.79)	
BMI (kg/m²), n (%)				<0.001
<18.5	90 (1.50)	3 (0.51)	87 (1.61)	
18.5-24.9	1514 (25.20)	61 (10.37)	1453 (26.81)	
25-29.9	1953 (32.51)	103 (17.52)	1850 (34.13)	
≥30	2451 (40.80)	421 (71.60)	2030 (37.45)	
Fasting glucose (mg/dl), median (IQR)	103.00 (96.00,115.00)	116.00 (102.00,144.00)	103.00 (96.00,113.00)	<0.001
HbA1c (%), median (IQR)	5.60 (5.30,6.00)	6.00 (5.60,6.80)	5.60 (5.30,5.90)	<0.001
Insulin (pmol/L), median (IQR)	60.78 (37.65,98.11)	106.80 (67.62,169.80)	57.78 (36.96,92.13)	<0.001
HOMA-IR, median (IQR)	2.67 (1.59,4.64)	5.85 (3.25,9.63)	2.54 (1.55,4.25)	<0.001
NAFLD, n (%)	2204 (36.37)	411 (69.19)	1793 (32.80)	<0.001
MASLD, n (%)	2199 (36.29)	410 (69.02)	1789 (32.73)	<0.001
T2DM, n (%)	907 (14.97)	208 (35.02)	699 (12.79)	<0.001
Antidiabetic medication				
GLP-1 receptor agonists	39 (0.64)	10 (1.68)	29 (0.53)	0.002
SGLT-2 inhibitors	52 (0.86)	13 (2.19)	39 (0.71)	0.001
Pioglitazone	39 (0.64)	9 (1.52)	30 (0.55)	0.012

NHANES: National Health and Nutrition Examination Survey; IQR: interquartile range; BMI: body mass index; HbA1c: hemoglobin A1c; HOMA-IR: homeostatic model assessment for insulin resistance; NAFLD: non-alcoholic fatty liver disease; MASLD: metabolic dysfunction-associated steatotic liver disease; T2DM: type 2 diabetes mellitus.

without, were predominantly aged ≥65 years (36.20%), male (57.74%), had a BMI of ≥30 kg/m^2 (71.60%), and were diagnosed with NAFLD (69.19%), MASLD (69.02%) and T2DM (35.02%). Additionally, fibrosis patients exhibited higher levels of fasting glucose, HbA1c, insulin, and HOMA-IR. Significant differences were observed across age, gender, race, BMI, fasting glucose, HbA1c, insulin, HOMA-IR, NAFLD, MASLD, and T2DM between the fibrosis and non-fibrosis populations.

After adjusting for potential confounders such as gender, age, race, and BMI, MASLD exhibited a significant association with fibrosis (β=1.029, 95%CI: 0.831-1.230) and T2DM (β=0.840, 95% CI: 0.674-1.006), as presented in Table II. Moreover, MASLD was linked to the biomarkers related to T2DM: fasting glucose (β=14.322, 95%CI: 11.411-17.233), HbA1c (β=0.454, 95%CI: 0.394-0.514), insulin (β=41.356, 95%CI: 30.077-52.634), and HOMA-IR (β=2.649, 95%CI: 1.968-3.330) (Table II).

Multivariable logistic regression analyses indicated that T2DM was significantly associated with fibrosis (adjusted OR=2.498, 95%CI: 2.034-3.062). Similarly, the biomarkers related to T2DM also exhibited a significant association with fibrosis: fasting glucose (adjusted OR=1.008, 95%CI: 1.005-1.011), HbA1c (adjusted OR=1.323, 95%CI: 1.240-1.410), insulin (adjusted OR=1.002, 95%CI: 1.001-1.003), and HOMA-IR (adjusted OR=1.035, 95%CI: 1.021-1.051) (Table III).

Mediation analyses, after adjusting for covariates, revealed that T2DM and its associated biomarkers significantly mediated the relationship between MASLD and fibrosis. Specifically, T2DM had a mediation effect of $\beta_{indirect}$ = 0.009 (95%CI: 0.006-0.010). T2DM-related biomarkers also showed significant mediation: fasting glucose ($\beta_{indirect}$ = 0.008, 95%CI:

0.005-0.010), HbA1c ($\beta_{indirect}$ = 0.008, 95%CI: 0.006-0.010), insulin ($\beta_{indirect}$ = 0.005, 95%CI: 0.002-0.010), and HOMA-IR ($\beta_{indirect}$ = 0.006, 95%CI: 0.002-0.010). The proportion of the association between MASLD and fibrosis that was mediated by these variables was 10.1% for T2DM, 9.99% for fasting glucose, 10.50% for HbA1c, 5.98% for insulin, and 7.28% for HOMA-IR. These findings are detailed in the Supplementary file and illustrated in Fig. 3.

To account for the impact of age, BMI, and antidiabetic medication, we conducted a subgroup analysis to examine whether the mediation effect of T2DM in the relationship of MASLD and fibrosis varied across different populations in Supplementary file. The association of MASLD with fibrosis was partly mediated by T2DM for 5.47% in the subjects of 18-44, 12.63% in the subjects of 45-64, and 10.17% in the subjects of ≥65. In addition, in the subgroup analysis of BMI groups, the mediation effect of T2DM was not significant in the subjects of underweight or normal weight, while T2DM mediated 17.74% of the total association between MASLD and fibrosis in the subjects of overweight, and 8.40% of that in subjects of obesity. Notably, in the subgroup analysis based on the use of antidiabetic medication, the mediation effect of T2DM was only significant in the subjects who did not use antidiabetic medication.

Sensitivity analyses were conducted to examine the robustness of the mediation results by changing the diagnostic criteria for MASLD from CAP ≥285 dB/m to CAP ≥274 dB/m. The mediation analyses showed that the results for T2DM (prop. mediated = 10.22%), fasting glucose (prop. mediated = 9.21%), HbA1c (prop. mediated = 9.92%), insulin (prop. mediated = 5.25%), and HOMA-IR (prop. mediated = 6.25%) remained statistically significant (Supplementary file).

Table II. Associations of MASLD with fibrosis, T2DM and T2DM-related biomarkers

Characteristic	Crude model		Adjusted model	
	β (95%CI)	p	β (95%CI)	p-value
Fibrosis	1.522 (1.340,1.706)	<0.001	1.029 (0.831,1.230)	<0.001
T2DM	1.131 (0.987,1.276)	<0.001	0.840 (0.674,1.006)	<0.001
Fasting glucose (mg/dl)	19.167 (16.495,21.839)	<0.001	14.322 (11.411,17.233)	<0.001
HbA1c (%)	0.599 (0.543,0.654)	<0.001	0.454 (0.394,0.514)	<0.001
Insulin (pmol/L)	63.092 (52.888,73.296)	<0.001	41.356 (30.077,52.634)	<0.001
HOMA-IR	3.856 (3.241,4.470)	<0.001	2.649 (1.968,3.330)	<0.001

Crude Model: unadjusted. Adjusted model: adjust for age, gender, race/ethnicity, BMI. For the rest of abbreviations see Table I.

Table III. Multivariable logistic regression model evaluating the effect of T2DM and T2DM-related biomarkers on the presence of fibrosis

Characteristic	Crude OR (95%CI)	p	Adjusted OR (95%CI)	p-value
T2DM	3.675 (3.047,4.423)	<0.001	2.498 (2.034,3.062)	<0.001
Fasting glucose (mg/dl)	1.010 (1.008,1.013)	<0.001	1.008 (1.005,1.011)	<0.001
HbA1c (%)	1.461 (1.376,1.550)	<0.001	1.323 (1.240,1.410)	<0.001
Insulin (pmol/L)	1.003 (1.002,1.004)	<0.001	1.002 (1.001,1.003)	<0.001
HOMA-IR	1.055 (1.038,1.075)	<0.001	1.035 (1.021,1.051)	<0.001

Crude Model: unadjusted. Adjusted model: adjust for age, gender, race/ethnicity, BMI. For the rest of abbreviations see Table I.
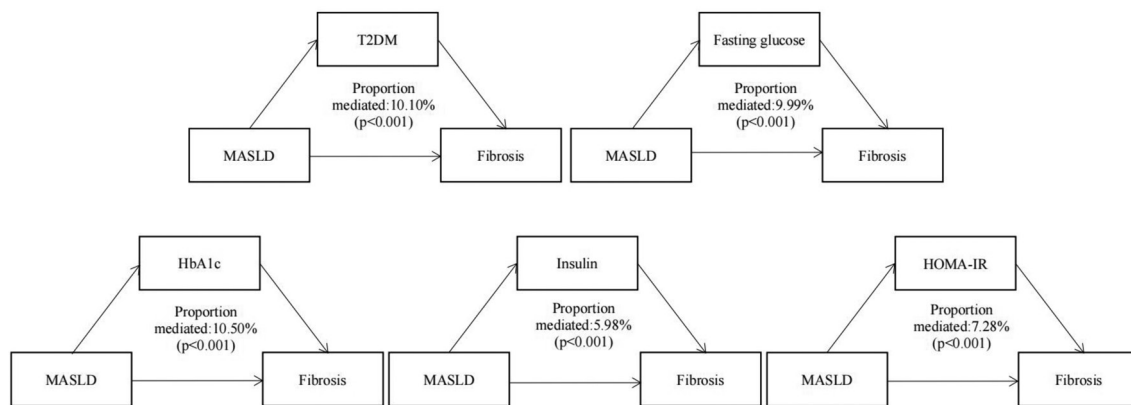
Fig. 3. Estimated proportion of the association between MASLD and fibrosis mediated by T2DM and T2DM-related biomarkers. For the rest of abbreviations see Table I.

DISCUSSION

In this cross-sectional study involving 6,060 adult participants, we evaluated the associations among MASLD, fibrosis, and T2DM and explored whether the relationship of MASLD and fibrosis was partly mediated by T2DM and T2DM-related biomarkers. Our results showed that MASLD, T2DM and related biomarkers, and fibrosis were pairwise associated. Moreover, T2DM might mediate the association of MASLD and fibrosis, explaining 10.1% of the total effect. Similar results were found for related biomarkers. In addition, the mediation effect of T2DM varied among different groups of age, BMI, and antidiabetic medication.

Current evidence suggested that the presence of comorbid illness factors, including T2DM, insulin resistance, dyslipidemia, obesity, hypertension, and hypopituitarism, accelerated the MASLD progression to fibrosis [3]. Meanwhile, the drug treatment for T2DM could improve advanced fibrosis [7]. Our results may confirm that T2DM may serve as a mediator in the association between MASLD and fibrosis. Previous studies have found the significance of MASLD in the onset of T2DM [24-26]. In our results, we found populations with MASLD had 2.30 times higher T2DM risk than those without MASLD. Our results also demonstrated that MASLD and T2DM were significantly associated with an increased risk of fibrosis, which was supported by prior studies [27-29].

The mediation effects were also evaluated to be statistically significant in the relationship of MASLD and fibrosis with fasting glucose, HbA1c, insulin, and insulin resistance as mediators. Prior studies showed that glucose metabolism dysregulation was associated with the progression of MASLD [30-34] and sodium-glucose co-transporter 2 inhibitors, which may improve blood glucose levels, were beneficial to MASLD and induced fibrosis [35]. In addition, MASLD patients with postprandial hyperinsulinemia tended to have a higher risk of advanced fibrosis [36]. Elevated levels of glucose and insulin often observed in individuals with MASLD have been documented to increase the expression of connective tissue growth factor (CTGF), which played a crucial role as an intermediary molecule implicated in the pathogenesis of chronic liver diseases characterized by fibrosis [37]. Similarly,

the role of insulin resistance in accelerating the fibrosis progression has been reported [38, 39]. Insulin resistance occurring in adipose tissue leads to heightened lipolysis within adipocytes, resulting in elevated levels of free fatty acids (FFAs) circulating in the bloodstream, particularly in the portal venous blood [40]. As a result, there was an increased uptake of FFAs by hepatocytes. Within the liver, FFAs promote lipid peroxidation, triggering the generation of highly reactive oxygen species, and activating the expression of proinflammatory cytokines like TNF-α. This, in turn, exacerbates necroinflammatory processes and contributes to the development of liver fibrosis [41].

Interestingly, our results of the mediation analysis indicated that the biomarkers of the glycemic status may serve as a more important mediator in the association of MASLD and fibrosis than the insulin level. This result was inconsistent with that of a recent study [42]. However, the previous evidence showed that the variability of blood glucose levels was a more significant factor than hyperinsulinemia in predicting the progression of hepatic fibrosis in MASLD [43]. Therefore, the significance of fasting glucose, HbA1c, and insulin in the fibrosis process of MASLD patients requires further investigations.

MASLD and T2DM were more likely to coexist in older adults and people of higher BMI [9]. In fact, previous studies have found a clear increase in incidents of advanced fibrosis linked to older age [44]. Meanwhile, obesity, as a key factor of fibrosis, has been discussed extensively [45]. According to the results of our subgroup analysis, we found that T2DM had a more mediating proportion of the total association in middle-aged and old people. Moreover, the mediation effect of T2DM was significant in adults of overweight and obesity. Therefore, these patients might need the systematic screening and more aggressive management strategies to prevent MASLD from progressing to fibrosis. Notably, we also found that the mediation effect of T2DM was not significant in adults using antidiabetic medication. This result indicated that the use of antidiabetic medication for different targets might be an effective therapeutic option for the MASLD patients with T2DM, which was aligned with previous studies [46, 47].

It was important to consider our findings within the framework of various assumptions that were integral to our analytical methodology. Our mediation analyses presupposed that

all potential confounding variables pertaining to the relationships of MASLD and fibrosis, MASLD and T2DM, as well as T2DM and fibrosis, were appropriately identified and adjusted for. In our study, we conducted a thorough assessment of the pertinent causal factors associated with MASLD, fibrosis, and T2DM, and made appropriate adjustments for major confounders based on a directed acyclic graph. Furthermore, sensitivity analyses were conducted to account for potential additional confounders.

To our knowledge, our study was the first to elucidate T2DM-related biomarkers that acted as mediators in the association between MASLD and fibrosis among the adult population. The study benefited from a substantial sample size and included a multiethnic population. However, our study bears some limitations. First, the reliance on self-reported diabetes may have introduced potential limitations in result validity. Second, our focus was solely on examining the mediation role of four T2DM-related biomarkers. The future research may include more biomarkers. Third, as for drugs and genetic diseases, due to the lack of clear data in the NHANES database, these factors have not been completely excluded in our study. Fourth, our mediation analyses were conducted within the framework of a cross-sectional study design, thereby impeding our ability to establish a causal relationship.

CONCLUSIONS

The association between MASLD and the risk of fibrosis was partly explained by T2DM and T2DM-related biomarkers. Gaining insight into the relative contribution of the T2DM pathway offered valuable information that could be utilized in population-level strategies aimed at mitigating the detrimental impact of MASLD on fibrosis. Further prospective and experimental studies were needed to validate its underlying mechanism and ascertain whether T2DM could serve as an effective target for the deceleration of the progression from MASLD to fibrosis.

Conflicts of interest: None to declare.

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