

# Study on correlation between hepatic fibrosis and fat indexes in patients with NAFLD and Type 2 diabetes mellitus

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## Research

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# Abstract

**Background:**To analyze the correlation between hepatic fibrosis and fat indexes in patients with non-alcoholic fatty liver disease (NAFLD) and type 2 diabetes mellitus(T2DM).

**Method:** Data of 135 NAFLD patients treated in the outpatient and inpatient department of gastroenterology of the Nantong Third People's Hospital Affiliated to Nantong University from January 2016 to December 2019 were collected and analyzed. The patients were divided into NAFLD group and NAFLD+T2DM group according to medical history, biochemical indexes and B-ultrasound examination results. The fat content and fibrosis degree were detected by FibroTouch instantaneous elastography. Risk factors and protective factors for hepatic fibrosis index were analyzed statistically.

**Results:** (1) Age, fibrosis index, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, body mass Index(BMI), low density lipoprotein(LDL), hemoglobin A1c(HbA1c) and C-reactive protein (CRP) levels in NAFLD + T2DM group were significantly higher than those in NAFLD group (  $P < 0.01$ , Table 1). HDL and peptide C in NAFLD + T2DM group were lower than those in NAFLD group. Step-by-step logistic regression suggested that ALT and LDLC were risk factors, and fibrosis index and peptide C were protective factors. (2) In NAFLD+T2DM group, HDLC and peptide C in the fibrosis subgroup were significantly decreased than compared with those in the non-fibrosis subgroup, and the creatinine, LDLC, HbA1C , Uric acid and BMI in the fibrosis subgroup were significantly increased compared with the non-fibrosis subgroup. (3) Linear regression analysis indicated that HDLC and HbA1C were risk factors and peptide C was protective factor.

**Conclusion:** Hepatic fibrosis is involved in the pathophysiological process of NAFLD and T2DM, and it is extreme importance to prevent hepatic fibrosis.

## 1. Background

Liver is an important metabolic organ, which exerts a crucial effect in regulating the homeostasis of glucose and lipid metabolism. Abnormal hepatic metabolism promotes insulin resistance, which is a common feature of metabolic diseases such as nonalcoholic fatty liver disease(NAFLD) and type 2 diabetes mellitus (T2DM). At present, the incidence rates of T2DM and NAFLD are constantly increasing, and the co-existence of the two diseases is not uncommon. NAFLD patients with insulin resistance have a higher risk for impaired fasting blood glucose and early diabetes; most T2DM patients experience non-alcoholic fatty liver (NAFL), non-alcoholic steatohepatitis (NASH) and other more serious liver complications, so there is a complex two-way relationship between NAFLD and T2DM. On the one hand, NAFLD leads to metabolic disorders characterized by insulin resistance and hyperinsulinemia. On the other hand, T2DM is a risk factor for NAFLD.

At present, there are few studies on the hepatic fibrosis degree and fat content of NAFLD patients with T2DM. This study aimed to objectively quantify hepatic fibrosis and fatty liver by FibroTouch technology and explore the possible risk factors.

## 2. Methods

### 2.1 Subjects

A total of 135 NAFLD patients treated in the outpatient and inpatient department of gastroenterology of the Nantong Third People's Hospital Affiliated to Nantong University from January 2016 to December 2019 were collected, of whom, there were 69 patients in the NAFLD group and 66 patients in NAFLD+T2DM group. A cross-sectional study was conducted, and the patients with viral hepatitis, autoimmune liver disease, drug-induced liver injury, alcoholic liver disease (alcohol intake) and incomplete data were excluded. The mean age of patients was ( $48.58 \pm 16.15$ ) years, and there were 79 males and 56 females.

### 2.2 Methods

The collected medical history data included: age and gender; alanine aminotransferase (ALT), aspartate aminotransferase (AST), body mass Index(BMI), creatinine, uric acid, total cholesterol(TC), triglyceride (TG), High-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C,) glycosylated hemoglobin(HbA1c), fasting Peptide C(FCP), C-reactive protein (CRP) (biochemical analyzer: Olympus AU2700). The hepatic fibrosis and fat indexes were measured by FibroTouch instantaneous elastography (model YZB / Su0437-204,Wuxi Haisi Kaier Medical Technology Co., Ltd.). (Hepatic fibrosis index

< 10 indicated mild hepatic fibrosis; 10-20 indicated moderate hepatic fibrosis; > 20 indicated severe hepatic fibrosis)

### 2.3 Diagnostic criteria

NAFLD was defined as follows: a. The imaging findings of liver met the diagnostic criteria of diffuse fatty liver and there was no other explanation; and / or b. the patients with metabolic syndrome related components had unexplained increase in serum ALT and / or AST, GGT continuously for more than half a year.

The working definition of T2DM was as follows: The fasting blood glucose was greater than or equal to 7.0mmol/L, and/ or 2-hour postprandial blood glucose was greater than or equal to 11.1mmol/L.

### 2.4 Statistical treatment

SPSS21.0 software was used for statistical analysis, and the measurement data in accordance with normal distribution were presented as  $\bar{x}+s$  and analyzed using *t*-test or single factor analysis of variance, and multivariate analysis were conducted using logistic regression,  $P < 0.05$  indicated that difference was statistically significant.

## 3. Results

### 3.1 Comparison of clinical data

Of 135 NAFLD patients, 135 (48.89%) had DM. Compared with patients in the NAFLD group, the patients in the NAFLD+T2DM group had higher age, fibrosis index, ALT, AST, creatinine, BMI, LDL, HbA1c and CRP, the differences were statistically significant ( $P < 0.05$ , Table 1), while HDL and peptide C in the NAFLD+T2DM group were lower than those in the NAFLD group.

Table 1. Comparison of clinical data between NAFLD + T2DM group and NAFLD group

Item	NAFLD	NAFLD+DM	*P
Age	39.1±16.67	48.6±16.2	0.001*
Hepatic fibrosis	13.7±6.10	15.8±5.43	0.038*
Hepatic steatosis	278.6±30.39	285.0±28.11	0.210
ALT	76.9±40.03	160.0±125.68	0.000*
AST	68.8±33.11	85.2±43.57	0.015*
CRE	57.3±13.37	66.0±12.37	0.000*
BMI	26.7±3.18	29.1±2.74	0.000*
TC	5.1±0.85	5.0±0.89	0.642
TG	2.0±0.87	2.0±0.78	0.904
HDL-C	1.2±0.27	1.08±0.23	0.000*
LDL-C	2.3±0.73	3.1±0.59	0.000*
Peptide C	3.6±0.79	2.3±1.21	0.000*
HbA1c	5.0±0.61	6.8±1.15	0.000*
Uric Acid	344.3±74.48	362.9±102.48	0.231
CRP	3.7±1.80	6.9±5.10	0.000*

### 3.2 Risk factor analysis

In order to determine whether the patients had DM dependent variables, 15 dependent variables such as hepatic fibrosis and steatosis were selected for logistic regression analysis. The results showed fibrosis index (OR = 0.683, 95% CI 0.487-0.959), ALT (OR = 1.050, 95% CI 1.106-1.084), LDLC (OR = 17.957, 95% CI 2.283-141.232), peptide C (OR = 0.047, 95% CI 0.006-0.368) and HbA1c (OR = 237.586, 95% CI 10.484-5384.259), and the differences were statistically significant. It was suggested that increased ALT, LDLC and HbA1c in NAFLD + T2DM group were the risk factors, while the fibrosis index and peptide C were the protective factors.

Table 2. Risk factors analysis of NAFLD +T2DM group

Variable	B	SE	Wald	P	OR(95%CI)
Hepatic fibrosis	-0.381	0.173	4.858	0.028	0.683[0.487-0.959]
ALT	0.048	0.017	8.621	0.003	1.050[1.106-1.084]
LDLC	2.888	1.052	7.533	0.006	17.957[2.283-141.232]
Peptide C	-3.050	1.046	8.505	0.004	0.047[0.006-0.368]
HbA1c	5.471	1.592	11.805	0.001	237.586[10.484-5384.259]

### 3.3 Clinical characteristics of patients with hepatic fibrosis

In the NAFLD + T2DM group, compared with the non-fibrosis subgroup, creatinine, LDLC, HbA1c, uric acid and BMI were significantly increased and HDLC and peptide C were significantly decreased in the fibrosis subgroup , and the differences were statistically significant.

Table 3. Comparison of clinical characteristics between subgroups in NAFLD + T2DM group

	Non-fibrosis	Uncertainty	Fibrosis	P
Age	43.44±13.12	49.07±17.03	15.27±15.60	0.581
Hepatic steatosis	273.44±30.45	285.67±27.29	290.0±29.03	0.37
ALT	162.67±55.56	155.88±134.38	169.8±136.11	0.934
AST	86±30.36	78.88±42.57	102.6±50.35	0.196
CRE	56.11±6.70	66.72±13.15	69.89±9.91	0.023 <sup>#</sup>
TC	5.18±0.63	4.91±0.86	5.10±1.13	0.615
TG	2.06±0.67	2.04±0.85	1.78±0.64	0.525
HDLC	1.26±0.13	1.09±0.23	0.92±0.22	0.001 <sup>#</sup>
LDLC	2.62±0.55	3.04±0.53	3.47±0.56	0.001 <sup>#</sup>
Peptide C	3.42±1.05	2.38±1.14	1.46±0.92	0.000 <sup>#</sup>
HbA1c	5.49±0.58	6.60±0.81	8.01±1.12	0.000 <sup>#</sup>
CRP	7.52±7.61	6.74±4.77	7.13±4.55	0.907
Uric Acid	341.11±85.24	345.64±99.91	424.4±100.65	0.028 <sup>#</sup>
BMI	27.38±2.01	28.83±2.11	30.82±3.76	0.005 <sup>#</sup>

### 3.4 Risk factors for hepatic fibrosis

Hepatic fibrosis was set as the dependent variable, and the independent variables with statistical differences between the subgroups were selected into the covariates for the ordinal multi factor linear regression analysis, and the results showed that HDLC and peptide C were protective factors, and HbA1c was risk factors. The differences were statistically significant ( $P < 0.05$ ). It was suggested that HDLC, peptide C and HbA1c could be used as important clinical indexes of hepatic fibrosis in patients with NAFLD and DM.

Table 4. Risk factors for hepatic fibrosis in the NAFLD +T2DM group

Variable	B	S.E.	T	P	OR(95%CI)
HDLC	5.223	3.601	-3.009	0.004	[-8.542-1.722]
Peptide C	-1.1710	0.365	-3.208	0.002	[-1.9-0.441]
HbA1c	2.78	0.379	7.331	0.000	[2.022-3.538]

## 4. Discussion

Recent studies have shown that with the prevalence of sedentary behavior and high-fat, high calorie diet, the incidence rates of metabolic diseases with similar pathological characteristics such as obesity, NAFLD and T2DM have been continuously increasing[1-2]. NAFLD related pathophysiology includes hepatic ectopic fat deposition, inflammation, endoplasmic reticulum (ER) stress and oxidative stress [3], all of which aggravate hepatic insulin resistance, promote metabolic disorders such as hyperglycemia and hyperlipidemia, and then lead to the occurrence of T2DM. Therefore, reducing the accumulation of liver lipid and improving the insulin resistance may be an effective way to prevent the progression of NAFLD to NAFLD complicated with T2DM. However, the exact mechanisms behind these pathological processes are not completely clear. Previous studies [4-6] have shown that NAFLD complicated with T2DM may aggravate the fibrosis degree. This study aimed to analyze the risk factors in two aspects, for example, whether NAFLD was complicated with T2DM and whether there was fibrosis in NAFLD complicated with T2DM. The results revealed that the incidence rate of T2DM in patients in the NAFLD +T2DM group was higher, reaching 48.89%, which was correlated with factors such as ALT, LDLC, peptide C and HbA1c, while the risk for fibrosis in the NAFLD +T2DM group was correlated with HDLC, peptide C and HbA1c.

At present, the diagnosis of NAFLD mainly depends on invasive liver biopsy. However, due to the large number of patients, invasive operation, high cost and other factors, it is difficult to carry out large-scale development of liver biopsy in China, which brings difficulties to the diagnosis of NAFLD, thus leading to the failure of timely diagnosis of such patients, further delaying the optimal treatment opportunity for patients. It is also of great clinical significance to advocate more active and systematic monitoring of NAFLD in T2DM patients in order to obtain potential early treatment [7]. In recent years, people have been trying to find an effective noninvasive way to diagnose NAFLD, for example, the study by Reis H et al has confirmed that as a marker for diagnosis of NASH, the sensitivity and specificity of the cytokeratin-18 (CK18) in peripheral blood cells is only 58% and 68% [8] and it is impossible to effectively find out those NASH patients who need treatment from NAFLD patients. In a study of Feldstein et al on the diagnostic value of circulating blood CK18 and its decomposed fragments for NAFLD, multivariate statistical analysis results showed that CK18 and its decomposed fragments can still be used as independent risk factors for predicting NASH after the interference of confounding factors are removed, and the area under the ROC curve for diagnosing NASH reaches 0.83 [9] but their methods and procedures are all complex. Recently, MR elastography has showed a high predictive value for excluding advanced fibrosis and a good accuracy for detecting NASH. MR elastography discriminated NASH from steatosis with a sensitivity of 94% and specificity of 73% but it is too expensive[10]. In this study, we intended to use the transient elastography to measure the hepatic fat and fibrosis indexes using real numbers, this is a technology based on the principle of ultrasound used for non-invasive detection of hepatic fibrosis and tissue elasticity degree. Because of its non-invasive and convenient detection method, compared with liver puncture, transient elastography technology has more advantages in the screening of hepatic fibrosis in the general population and the long-term follow-up of patients. In the presentation of results, FibroTouch instantaneous elastography uses specific numbers to indicate the specific degree of hepatic

fibrosis and fatty liver, and quantify mild, moderate and severe fibrosis degrees that were relatively blurred during the previous liver puncture into specific numbers, this makes it easy for doctors to make clear diagnosis and facilitate patient reexamination.

At present, increased transaminase in most clinical cases are caused by NAFLD, its incidence is much higher than that caused by alcohol, virus and biliary diseases. In this study, the levels of ALT, AST and CRP in NAFLD +T2DM group were significantly higher than those in NAFLD group, and the differences showed a statistical significance. It was considered to be related to the increased oxidative stress and inflammatory response in the NAFLD +T2DM group. The CRE level in the NAFLD +T2DM group was significantly higher than that in the NAFLD group, and the difference was statistically significant, indicating that renal function was affected. Liver is the hub for glucose and lipid metabolism. Liver structure and function are destroyed during hepatic fibrosis, which leads to abnormal blood glucose and lipid. In this study, TC and TG in the NAFLD +T2DM group showed no significant difference, HDL and LDL in the NAFLD +T2DM group showed a significant difference (the decrease in TG may be related to the decrease in endogenous lipoprotein synthesis). Obesity (increased BMI), abnormal lipid metabolism and glucose tolerance are the causes for NAFLD fibrosis. After hepatic fibrosis, the liver intake of glucose is reduced or the hepatic glycogen synthesis is impaired, which further aggravates hepatic fibrosis, and thus leading to a vicious circle. A related study[11-13]suggested that the hepatic fibrosis degree in NAFLD + T2DM group is higher than that in the NAFLD group, but this study suggested that hepatic fibrosis can be used as a protective factor for NAFLD to progress to NAFLD complicated with T2DM, which urges us to consider two possibilities: 1. Whether there is a positive correlation between the results of FibroTouch instantaneous elastography and results of pathological examination of liver puncture biopsy during hepatic fibrosis degree measurement; 2. The occurrence of hepatic fibrosis is actually a protective measure for the progression of NAFLD to NAFLD complicated T2DM , and the causes need to be verified in further studies.

The relationship between NAFLD and T2DM is complex and bidirectional. NAFLD provides a necessary biological environment for the progression of T2DM [14], and the presence of T2DM increases the risk of liver diseases [15], which may progress into NASH, cirrhosis or even HCC [16].

At present, it is not common to screen the liver related complications in T2DM patients, and the liver is still a potentially neglected organ in the progression of chronic metabolic diseases. However, the sample size of this study is small, and the data may be biased. We will continue to collect and sort out the data in the future work to further understand the correlation between liver fat and fibrosis indexes in patients with NAFLD and T2DM, which will provide scientific basis for further exploration of the treatment of metabolic diseases.

## Conclusion

Hepatic fibrosis is involved in the pathophysiological process of NAFLD and T2DM, and it is extreme importance to prevent hepatic fibrosis.

## Abbreviations

NAFLD

Non-alcoholic fatty liver disease

T2DM

Type 2 diabetes mellitus

BMI

Body mass Index

ALT

Alanine aminotransferase

AST

Aspartate aminotransferase

LDL

Low density lipoprotein

HDL

High density lipoprotein

HbA1c

Hemoglobin A1c

CRP

C-reactive protein

NASH

Non-alcoholic steatohepatitis

## Declarations

Acknowledgments

None.

Authors' contributions

Jianguo Shao was the main coordinator of the project and was responsible for the study design. Lingyan Zhang drafted the manuscript of the present paper. Xiang Ji was involved in the supervising of data collection and stratification. Jiajia Shi contributed to data assembly and analysis. TingYu Peng, Dengfu Yao and Min Yao contributed with manuscript revision. All authors contributed intellectually to this manuscript and have approved this final version.

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#### Availability of data and materials

The data in the current paper are publicly available .

#### Ethics approval and consent to participate

Not applicable.

#### Consent for publication

Not applicable.

#### Competing interests

The authors declare that they have no competing interests.

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