ORIGINAL RESEARCH

Evaluating the Significance of Fasting C-peptide in Conjunction with the Insulin Resistance Index for Assessing Hepatic Fibrosis in Patients with Type 2 Diabetes Mellitus and Nonalcoholic Fatty Liver Disease

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ABSTRACT

Background • Non-alcoholic fatty liver disease (NAFLD) has reached pandemic proportions globally, particularly affecting individuals with type 2 diabetes mellitus (T2DM).

Objective • Our study aims to elucidate the diagnostic value of fasting C-peptide in combination with insulin resistance for assessing hepatic fibrosis in patients with T2DM and comorbid NAFLD.

Design • This was a retrospective study.

Setting • The study was conducted at the Affiliated Hospital of Integrated Traditional Chinese and Western Medicine, Nanjing University of Chinese Medicine.

Participants • The research involved 76 type 2 diabetes mellitus patients with nonalcoholic fatty liver disease, diagnosed at our hospital from April 2020 to October 2022. Patients were categorized into the non-progressive hepatic fibrosis group (n = 64) and progressive hepatic fibrosis group (n = 12) based on fibrosis-4 value.

Interventions • General data, systolic/diastolic blood pressure, fasting plasma glucose, fasting C-peptide, fasting insulin, glycosylated hemoglobin, uric acid, triglyceride, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, aspartate transaminase, alanine transaminase, and γ-glutamyl transferase were collected. Insulin resistance was calculated using a designated formula.

Primary Outcomes Measures • The predictive impact of fasting C-peptide in combination with insulin resistance was evaluated through receiver operating characteristic curves.

Results • The age, body mass index, fasting C-peptide, fasting insulin, aspartate transaminase, and insulin resistance showed a significant increase in the progressive hepatic fibrosis group compared to the non-progressive group ($P = .006$, $P = .014$, $P < .001$, $P < .001$, $P = .004$, and $P = .021$). The combination's sensitivity demonstrated an elevation compared to fasting C-peptide or insulin resistance alone ($P = .005$).

Conclusions • Fasting C-peptide in combination with insulin resistance proves to have a substantial predictive impact on hepatic fibrosis in type 2 diabetes mellitus patients with nonalcoholic fatty liver disease, holding valuable clinical diagnostic potential. (*Altern Ther Health Med.* [E-pub ahead of print.])

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a form of liver injury resulting from metabolic stress, independent of alcohol consumption, with its primary manifestation being the accumulation of fat in hepatocytes exceeding 5%.[1,2] NAFLD encompasses two main types: primary, associated with insulin resistance (HOMA-IR) and genetic predisposition, and secondary, caused by specific underlying factors.[3]

Excessive weight gain and nutritional overconsumption leading to excess weight, along with fatty liver associated with metabolic syndrome conditions such as obesity, diabetes, hyperlipidemia, and cryptogenic fatty liver, belong to the category of primary NAFLD.[4] On the other hand, fatty liver resulting from malnutrition, total parenteral nutrition, rapid weight loss post-bariatric surgery, and exposure to drugs,

environmental toxins, or industrial toxicological substances fall under the category of secondary NAFLD.[5]

The majority of NAFLD patients are asymptomatic, although some may experience fatigue, dyspepsia, dull pain in the liver area, and non-specific symptoms such as liver and spleen enlargement.[6] With the development of the social economy and changes in people's living and eating habits in recent years, the incidence of NAFLD has shown a steady increase, particularly among the population with type 2 diabetes mellitus (T2DM), where its prevalence is significantly higher.[7,8]

Prior research has indicated that diabetes serves as the foremost predictor of exacerbation and the progression of hepatic fibrosis in patients with NAFLD.[9] Furthermore, the ongoing advancement of hepatic fibrosis in NAFLD patients increases the risk of diabetes-related microvascular complications, all-cause liver mortality, and the development of liver cancer.[10,11] Clinical experience from previous studies underscores that the early diagnosis of hepatic fibrosis in individuals with T2DM complicated by NAFLD is crucial for mitigating chronic complications of diabetes and inhibiting the progression of hepatic fibrosis.

HOMA-IR is typically characterized by diminished sensitivity and reactivity in glucose metabolism mediated by islet beta cells, inhibiting the conversion of liver glucose to glucagon. It plays a significant pathophysiological role in the development of T2DM.[12] C-peptide, also known as the linker peptide, is secreted by pancreatic beta cells and shares a precursor with insulin. Due to its resistance to rapid degradation by the liver, the measurement of C-peptide serves as an indirect measure of insulin levels, offering an accurate reflection of the functioning of islet cells.[13] Fasting C-peptide and HOMA-IR exhibit associations with the progression of hepatic fibrosis.[14]

However, there are limited studies on the simultaneous use of both indicators to assess the status of hepatic fibrosis in patients with T2DM complicated by NAFLD. Therefore, our study aimed to explain the diagnostic value of fasting C-peptide in combination with HOMA-IR for assessing hepatic fibrosis in patients with T2DM complicated by NAFLD, providing valuable insights for early detection and intervention.

MATERIALS AND METHODS

Study Design

A retrospective study design was adopted, and a total of 76 T2DM patients complicated by NAFLD and diagnosed in our hospital from April 2020 to October 2022 were prospectively included in this study. This research received review and approval from the medical ethics committee of our hospital. Both patients and their family members were provided with an understanding of the research content and signed informed consent.

Inclusion and Exclusion Criteria

Inclusion criteria were as follows: (1) compliance with the diagnostic criteria for T2DM revised by the World Health Organization in 1999;[15] (2) compliance with the diagnostic benchmark for NAFLD in the Guidelines for the *Diagnosis of Nonalcoholic Fatty Liver Disease issued by the Liver Diseases,* Branch of the Chinese Medical Association in 2010; and (3) Patients' age ranged from 18 to 75 years.

Exclusion criteria were as follows: (1) Metabolic diseases impairing liver function; (2) Previous history of alcohol consumption exceeding recommended limits (male: > 140 g/week; female: > 70 g/week); (3) Gestational diabetes, type 1 diabetes mellitus, or other types of diabetes; (4) Complications with drug-induced liver disease, hepatobiliary obstruction, or biliary tract infection; (5) Hypothyroidism, hyperthyroidism, tumors, or hematologic diseases; (6) Recent use of drugs affecting insulin levels, C-peptide secretion, or blood lipid levels; (7) Fibrosis index based on fibrosis-4 (FIB-4) below 1.3.

Patient Demographics

A total of 76 patients were included (48 males and 28 females), aged 38 to 74 years, with a mean age of (53.47 ± 12.65) years. Based on FIB-4, patients with values of 1.3-2.67 kPa were included in the non-progressive hepatic fibrosis group (n = 64), while those with values above 2.67 kPa were included in the progressive hepatic fibrosis group (n = 12).

Data Collection

Clinical Data. Patient demographic information, including age, gender, body mass index (BMI), and the duration of T2DM, was collected.

Blood Pressure Measurement. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured after a half-hour of rest.

Laboratory Measurements. Venous blood samples were drawn after an 8-hour fast for the assessment of fasting plasma glucose (FPG), fasting C-peptide, fasting insulin (FINS), glycosylated hemoglobin (HbA1c), uric acid (UA), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density-lipoprotein cholesterol (LDL-C), aspartate transaminase (AST), alanine transaminase (ALT), and γ-glutamyl transferase (γ-GGT).

HOMA-IR Calculation. The Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) was calculated using the formula: HOMA-IR = (FINS * FPG) / 22.5.[16]

Statistical Analysis

Statistical analysis was conducted using SPSS 21.0 software (IBM Corp., Armonk, NY, USA). Counting data were expressed as [n (%)], and between-group comparisons were performed using the χ^2 test. Measurement data with a normal distribution were presented as mean ± standard deviation ($\bar{x} \pm s$), and inter-group comparisons were conducted using the t test. For measurement data with a non-normal distribution, the expression utilized the median and quartile [M (P25, P75)], and inter-group comparisons were performed using non-parametric tests. The predictive efficacy of fasting C-peptide in conjunction with HOMA-IR was evaluated through receiver operating characteristic (ROC) curves. A significance level of $P < .05$ indicated a statistical difference.

RESULTS

Univariate Analysis of Factors Affecting Hepatic Fibrosis in T2DM Patients Complicated by NAFLD

In the progressive hepatic fibrosis group, age, BMI, fasting C-peptide, FINS, AST, and HOMA-IR exhibited a significant increase compared to the non-progressive hepatic fibrosis group, demonstrating statistical significance ($P = .006$, $P = .014$, $P < .001$, $P < .001$, $P = .004$, and $P = .021$). No significant differences were observed in gender, SBP, DBP, FPG, HbA1c, UA, HDL-C, LDL-C, T2DM duration, TG, ALT, and γ-GGT between both groups ($P = 0.928$, $P = .564$, $P = .875$, $P = .205$, $P = .257$, $P = .911$, $P = .299$, $P = .083$, $P = .768$, $P = .605$, $P = .564$, and $P = .248$); refer to Table 1. This result indicates that patients' age, BMI, fasting C-peptide, FINS, AST, and HOMA-IR may serve as risk factors influencing hepatic fibrosis in T2DM patients complicated by NAFLD.

Predictive Efficacy of Fasting C-peptide or HOMA-IR Alone for Hepatic Fibrosis in T2DM Patients Complicated by NAFLD

The predictive efficacy of fasting C-peptide or HOMA-IR alone was assessed through ROC curves. The AUC for fasting C-peptide was 0.841 (95% CI: 0.647-1.00), with a cutoff value of 2.66 ng/ml. It exhibited a diagnostic sensitivity of 83.30%, a specificity of 96.90%, and an accuracy of 90.10%. In comparison, the AUC for HOMA-IR was 0.789 (95% CI: 0.599-0.979), with a cutoff value 5.87. It demonstrated a diagnostic sensitivity of 75.00%, a specificity of 95.30%, and an accuracy of 90.30%, refer to Figure 1.

Predictive Efficacy of the Combination of Fasting C-peptide and HOMA-IR for Hepatic Fibrosis in T2DM Patients Complicated by NAFLD

The predictive efficacy of the combination of fasting C-peptide and HOMA-IR was assessed through ROC curves. The AUC for the combination was 0.868 (95% CI: 0.712-1.00), with a diagnostic sensitivity of 92.00%, specificity of 97.10%, and an accuracy of 92.00%, refer to Figure 2.

Comparative Analysis of Predictive Efficacy: Fasting C-peptide, HOMA-IR Alone, and Their Combination in T2DM Patients with NAFLD

No significant differences were observed in specificity and accuracy between fasting C-peptide or HOMA-IR alone and their combination ($P = .686$ and $P = .854$). However, the combination demonstrated an elevated sensitivity compared to fasting C-peptide or HOMA-IR alone, indicating statistical significance ($P = .005$); refer to Table 2.

DISCUSSION

Patients with T2DM are susceptible to NAFLD. T2DM stands as an independent risk factor contributing to the worsening of NAFLD, with a likelihood of detecting NAFLD surpassing 70% among T2DM patients. Within this population, approximately 17%-37% of patients progress to develop hepatic fibrosis, representing a probability two to four times higher than that of the normal population.[17]

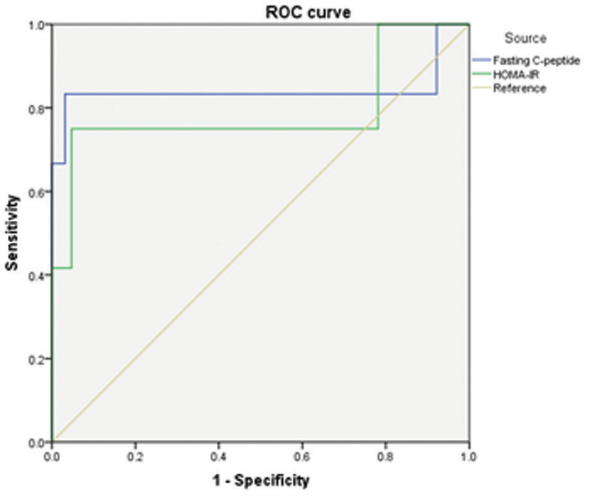
Table 1. Factors Affecting Hepatic Fibrosis In T2DM Patients Complicated by NAFLD

Variables	n	Non-Progressive Hepatic Fibrosis Group	Progressive Hepatic Fibrosis Group	χ^2/t	*P* value
Age (year)	76	50.82 ± 9.68	58.81 ± 12.88[a]	3.395	.006
Gender (male/female)	76	54/10	10/2	0.008	.928
BMI (kg/m^2)	76	25.52 ± 2.98	27.84 ± 3.35[a]	2.91	.014
T2DM Course (year)	76	4.65 (0.96, 8.95)	3.61 (0.99, 9.41)	0.333	.564
SBP (mmHg)	76	133.63 ± 17.87	137.75 ± 25.22	0.161	.875
DBP (mmHg)	76	83.55 ± 10.33	84.67 ± 13.65	1.348	.205
FPG (mmol/L)	76	10.52 ± 4.22	11.63 ± 4.60	1.196	.257
Fasting C-Peptide (ng/ml)	76	1.67 (1.09, 2.63)	3.15 (1.86, 3.85)[a]	12	<.001
FINS (mU/L)	76	15.49 (8.96, 18.95)	24.01 (15.15, 34.85)[a]	12	<.001
HbA1c (%)	76	9.52 ± 2.18	9.05 ± 2.40	0.115	.911
UA (mol/L)	76	334.69 ± 91.25	354.27 ± 95.12	1.091	.299
TG (mmol/L)	76	2.49 (1.52, 3.45)	2.69 (1.76, 3.97)	3	.083
HDL-C (mmol/L)	76	1.07 ± 0.30	1.09 ± 0.33	0.302	.768
LDL-C (mmol/L)	76	2.84 ± 1.27	2.61 ± 1.16	0.533	.605
AST (U/L)	76	27.10 (18.00, 37.00)	36.54 (24,50, 45.50)[a]	8.333	.004
ALT (U/L)	76	32.89 (19.50, 45.50)	33.71 (20.50, 46.00)	0.333	.564
γ-GGT (U/L)	76	49.61 (27.50, 62.00)	54.39 (24.50, 71.00)	1.333	.248
HOMA-IR	76	2.99 (1.42, 6.29)	3.96 (2.75, 7.65)[a]	5.333	.021

[a]$P < .001$, compared with the Non-progressive hepatic fibrosis group.

Abbreviations: BMI, body mass index; T2DM, type 2 diabetes mellitus; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; FINS, fasting insulin; HbA_{1c}, glycosylated hemoglobin; UA, uric acid; TG, triglyceride; HDL-C, high-density-lipoprotein cholesterol; LDL-C, low-density-lipoprotein cholesterol; AST, aspartate transaminase; ALT, alanine transaminase; γ-GGT, γ-glutamyl transferase; HOMA-IR, insulin resistance.

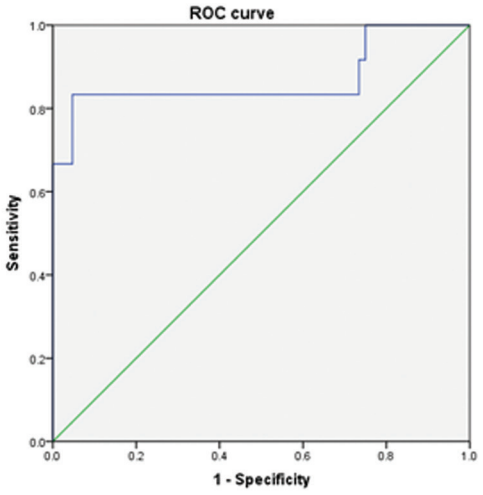
Figure 1. ROC Curve Analysis of Fasting C-peptide and HOMA-IR Alone in T2DM Patients Complicated by NAFLD



Note: The ROC curve illustrates the receiver operating characteristic of fasting C-peptide and HOMA-IR as individual predictors for hepatic fibrosis in individuals with type 2 diabetes mellitus (T2DM) complicated by nonalcoholic fatty liver disease (NAFLD). ROC, standing for receiver operating characteristic, represents the trade-off between sensitivity and specificity. HOMA-IR represents insulin resistance.

This study identified 15.79% of T2DM patients complicated by NAFLD with progressive hepatic fibrosis, aligning with prior research findings. Our study focused on T2DM patients with confirmed NAFLD. We assessed hepatic fibrosis using test results, clinical data, and experience. The study clarified the significance of combining fasting C-peptide with HOMA-IR for evaluating hepatic fibrosis in T2DM patients with NAFLD. This insight may serve as a foundational reference for the early diagnosis of hepatic fibrosis degree in these patients.

Figure 2. ROC Curve Analysis of the Combination of Fasting C-peptide and HOMA-IR in T2DM Patients Complicated by NAFLD



Note: The ROC curve depicts the receiver operating characteristic (ROC) of the combination of fasting C-peptide and HOMA-IR in predicting hepatic fibrosis in individuals with type 2 diabetes mellitus (T2DM) complicated by nonalcoholic fatty liver disease (NAFLD). The analysis evaluates the joint diagnostic performance of fasting C-peptide and HOMA-IR, offering insights into their combined efficacy as predictors for hepatic fibrosis in this specific population.

Table 2. Predictive Efficacy of Fasting C-peptide or HOMA-IR Alone and Their Combination in T2DM Patients Complicated by NAFLD

Groups	AUC	95% CI	Cut-off	Sensitivity	Specificity	Accuracy
Fasting C-peptide (ng/ml)	0.841	0.647-1	2.66	83.30%	96.90%	90.10%
HOMA-IR	0.789	0.599-0.979	5.87	75.00%	95.30%	90.30%
Combination	0.868	0.712-1	/	92.00%	97.10%	92.00%
χ^2	/	/	/	10.416	0.755	0.315
P value	/	/	/	.005	.686	.854

Abbreviations: AUC, area under the curve; CI, confidence interval; HOMA-IR, insulin resistance.

A liver biopsy serves as the gold standard for examining liver fibrosis. However, its invasiveness makes it unsuitable for early detection.[18] FIB-4, a widely used noninvasive clinical examination, offers high accuracy and greater patient acceptance.[19] In our study, FIB-4 proved to be a feasible criterion for assessing the progression of hepatic fibrosis in T2DM patients with NAFLD.

In this context, the progressive hepatic fibrosis group exhibited significant elevations in age, BMI, fasting C-peptide, FINS, AST, and HOMA-IR compared to the non-progressive hepatic fibrosis group. This result suggests that patients' age, BMI, fasting C-peptide, FINS, AST, and HOMA-IR could serve as risk factors influencing hepatic fibrosis in T2DM patients complicated by NAFLD.

As patients age, their metabolic capacity decreases, increasing the likelihood of hepatic fibrosis. Moreover, higher BMI correlates with elevated liver fat content, liver stiffness, and pancreatic fat content. These factors positively correlate with the development of hepatic fibrosis in NAFLD patients.[20,21]

C-peptide, a peptide hormone, is released simultaneously with equal moles of insulin during the cleavage and hydrolysis of human proinsulin molecules. As a result, it can be utilized for measuring insulin secretion and evaluating the activity of residual islet B cells.[22] Currently, there are varying conclusions regarding the impact of fasting C-peptide on the development of hepatic fibrosis in NAFLD patients. Some reports suggest that C-peptide has a negative correlation with the progression of hepatic fibrosis and a positive correlation with the inflammatory development of liver steatosis.[23]

Some studies suggest that C-peptide can delay the occurrence of complications in T2DM patients.[24] In this context, fasting C-peptide emerged as a risk factor influencing hepatic fibrosis in T2DM patients complicated by NAFLD. This may be attributed to the C-peptide representing the spontaneous insulin level secreted by the human body. In early NAFLD patients, excessive insulin secretion can lead to hyperinsulinemia, stimulating hepatocytes to secrete the matrix excessively, thereby accelerating the development of hepatic fibrosis.[25]

C-peptide, acting as a signaling regulatory enzyme, modulates the nuclear factor-kappa B (NF-κB)-Jun N-terminal kinase (JNK) ceramide pathway. It enhances the transcription and expression of glycogenic proteins in the human body, influencing cell apoptosis, nuclear division, and the production of proinflammatory cytokines. Additionally, it accelerates hepatocyte apoptosis and lysis.[26] Moreover, it has been reported that increased fasting insulin sensitivity (FIS) is closely related to patients with NAFLD.[27]

Ryan et al.[28] have indicated that FIS is elevated in individuals with non-alcoholic fatty liver disease. AST, a commonly applied indicator of liver function in the body, is typically present in mitochondria. AST levels can increase when hepatocytes undergo inflammation, leading to hepatocyte damage and necrosis.[29] Hyperinsulinemia and HOMA-IR can upregulate insulin-like growth factor-1 (IGF-1) and insulin levels. Elevated IGF-1 can activate the Wnt/β-catenin signaling cascade, resulting in hepatic fibrosis and carcinogenesis.[30]

Our study also revealed that the sensitivity of the combination of fasting C-peptide and HOMA-IR was elevated relative to those of fasting C-peptide or HOMA-IR alone. This finding suggests that the combination of fasting C-peptide and HOMA-IR may significantly enhance the diagnostic sensitivity for hepatic fibrosis in T2DM patients complicated by NAFLD. These findings align with previous studies.[31-33]

Study Limitations

Our study has certain limitations. Firstly, it is a single-center study with participants exclusively from the same hospital, introducing potential bias into the results. Secondly, the sample size of enrolled patients was small, and the unavailability of liver biopsy for large-scale diagnosis impacted the study outcomes. Therefore, future research should include large-scale and multi-center studies to validate our findings further.

CONCLUSION

In conclusion, our study highlights several critical risk factors contributing to hepatic fibrosis in patients with T2DM complicated by NAFLD. The analysis reveals that age, BMI, fasting C-peptide, FINS, AST, and HOMA-IR collectively play pivotal roles in influencing the development of hepatic fibrosis in this specific population. Our findings underscore the significant predictive impact of combining fasting C-peptide with HOMA-IR in assessing hepatic fibrosis in T2DM patients with NAFLD. This combined approach demonstrates a substantial elevation in diagnostic sensitivity and holds valuable potential for clinical diagnosis. This comprehensive understanding of the relationship between these risk elements provides essential insights for clinicians and researchers, emphasizing the need for a holistic approach to evaluating and managing hepatic fibrosis in individuals with T2DM and NAFLD.

CONFLICTS OF INTEREST

The authors report no conflict of interest.

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AVAILABILITY OF DATA AND MATERIALS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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