

## Retrospective Cohort Study

## Histopathological differences utilizing the nonalcoholic fatty liver disease activity score criteria in diabetic (type 2 diabetes mellitus) and non-diabetic patients with nonalcoholic fatty liver disease

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### Abstract

**AIM:** To study clinical and histopathological features of nonalcoholic fatty liver disease (NAFLD) in patients with and without type 2 diabetes mellitus (T2DM) using updated nonalcoholic steatohepatitis clinical research network (NASH-CRN) grading system.

**METHODS:** We retrospectively analyzed data of 235 patients with biopsy proven NAFLD with and without T2DM. This database was utilized in the previously published study comparing ethnicity outcomes in NAFLD by the same corresponding author. The pathology database from University of Chicago was utilized for enrolling consecutive patients who met the criteria for NAFLD and their detailed clinical and histopathology findings were obtained for comparison. The relevant clinical profile of patients was collected from the Electronic Medical Records around the time of liver biopsy and the histology was read by a single well-trained histopathologist. The updated criteria for type 2 diabetes have been utilized for analysis. Background data of patients with NASH and NAFLD has been included. The mean differences were compared using  $\chi^2$  and *t*-test along with regression analysis to evaluate the predictors of NASH and advanced fibrosis.

**RESULTS:** Patients with NAFLD and T2DM were significantly older (49.9 *vs* 43.0,  $P < 0.01$ ), predominantly female (71.4 *vs* 56.3,  $P < 0.02$ ), had higher rate of metabolic syndrome (88.7 *vs* 36.4,  $P < 0.01$ ), had significantly higher aspartate transaminase (AST)/alanine transaminase (ALT) ratio (0.94 *vs* 0.78,  $P < 0.01$ ) and Fib-4 index (1.65 *vs* 1.06,  $P < 0.01$ ) as markers of NASH, showed higher mean NAFLD activity score (3.5 *vs* 3.0,  $P = 0.03$ ) and higher mean fibrosis score (1.2 *vs* 0.52,  $P < 0.01$ ) compared to patients with NAFLD without T2DM. Furthermore, advanced fibrosis (32.5 *vs* 12.0,  $P < 0.01$ ) and ballooning (27.3 *vs* 13.3,  $P < 0.01$ ) was significantly higher among patients with NAFLD and T2DM compared to patients with NAFLD without T2DM. On multivariate analysis, T2DM was independently associated with NASH (OR = 3.27, 95%CI: 1.43-7.50,  $P < 0.01$ ) and advanced fibrosis (OR = 3.45, 95%CI: 1.53-7.77,  $P < 0.01$ ) in all patients with NAFLD. There was a higher rate of T2DM (38.1 *vs* 19.4,  $P < 0.01$ ) and cirrhosis (8.3 *vs* 0.0,  $P = 0.01$ ) along with significantly higher mean Bilirubin (0.71 *vs* 0.56,  $P = 0.01$ ) and AST (54.2 *vs* 38.3,  $P < 0.01$ ) and ALT (78.7 *vs* 57.0,  $P = 0.01$ ) level among patients with NASH when compared to patients with steatosis alone. The mean platelet count (247 *vs* 283,  $P < 0.01$ ) and high-density lipoprotein cholesterol level (42.7 *vs* 48.1,  $P = 0.01$ ) was lower among patients with NASH compared to patients with steatosis.

**CONCLUSION:** Patients with NAFLD and T2DM tend to have more advanced stages of NAFLD, particularly advanced fibrosis and higher rate of ballooning than patients with NAFLD without T2DM.

**Key words:** Non-alcoholic steatohepatitis; Non-alcoholic fatty liver disease; Advanced fibrosis; Non-alcoholic fatty liver disease activity score; Type 2 diabetes; Liver biopsy

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**Core tip:** This retrospective cohort study shows that type

2 diabetes mellitus (T2DM) is a reliable predictor for both nonalcoholic steatohepatitis (NASH) and advanced fibrosis. Patients with nonalcoholic fatty liver disease (NAFLD) and uncontrolled T2DM tend to have advanced fibrosis and higher rate of ballooning histologically. It is important to recognize the differences between composite NAS and individual histological features while interpreting liver biopsies among NAFLD patients. Early diagnosis of NASH and advanced fibrosis in patients with NAFLD has important clinical significance especially to prevent further progression of liver disease to cirrhosis, hepatocellular carcinoma and other related complications. Thus, optimization of risk factors for NAFLD such as metabolic syndrome, uncontrolled T2DM and dyslipidemia is of paramount importance.

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## INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) includes a histological spectrum of liver diseases ranging from simple steatosis to nonalcoholic steatohepatitis (NASH) and the latter histological entity can progress to cirrhosis in patients without significant alcohol consumption<sup>[1]</sup>. NAFLD is considered to be the hepatic manifestation of metabolic syndrome (MS)<sup>[2]</sup>. Type 2 diabetes mellitus (T2DM) is not only associated with NAFLD, but has also been shown to be an independent risk factor for the development of NASH<sup>[2-4]</sup>. While screening for diabetes and other risk factors for NAFLD is easily performed, evaluation and management of NAFLD is still challenging. In spite of having a well-defined understanding of the stages of NAFLD progression, the exact pathogenesis is still unclear<sup>[5]</sup>.

Despite the availability of various non-invasive tools, liver biopsy is still regarded as the gold standard for accurate measurement of histopathological features of NAFLD<sup>[6,7]</sup>. Identifying the unique histopathological features of NAFLD among patients with T2DM is not only important to stage the disease progression but also help us understand the impact of diabetes in progression of NAFLD. At present, there are only few studies describing histopathological differences among NAFLD patients with and without T2DM in a multiethnic United States population cohort<sup>[5,8]</sup>. Moreover, these studies utilized the Brunt scoring system<sup>[8,9]</sup> and were limited in sample size<sup>[8]</sup> or included pooled data from various centers<sup>[5]</sup>.

Therefore, the primary aim of our study was to evaluate and compare the clinical, laboratory and detailed histological findings using the updated NASH

clinical research network (CRN) scoring system among biopsy proven NAFLD patients with and without T2DM using a single histopathologist from a major tertiary health care center. We also explored the risk of NASH and advanced fibrosis in both groups in addition to comparing the background data of NAFLD and NASH separately.

## MATERIALS AND METHODS

### Study design

The pathology database from the University of Chicago Medical Center (UCMC) containing the terms "steatosis", "steatohepatitis" and/or "fat" from June 1, 1995 to June 30, 2005 was retrospectively analyzed and 683 positive biopsy reports were consecutively identified for further analysis. UCMC's computerized medical records were then retrospectively reviewed to obtain patient-related demographic, clinical, and laboratory data. Patients either lacking adequate information and/or having presence of other concomitant liver diseases including hepatitis B and C, iron overload, medication-related steatosis, significant alcohol use (current daily alcohol consumption of 40 g/d or more in males and 20 g/d or more in females) and liver transplant were excluded. Data on T2DM and MS were collected based upon American Diabetes Association<sup>[10]</sup> and National Cholesterol Education Program ATP III criteria<sup>[11]</sup>. Obesity was defined as body mass index (BMI)  $\geq 30$  kg/m<sup>2</sup> since data on waist circumference measures of central obesity were unavailable<sup>[12]</sup>. Out of the 683 patients with fatty liver histology, only 238 patients were found to be meeting the criteria for NAFLD and on further review, 3 patients had inadequate data regarding presence or absence of T2DM, thereby leaving a total of 235 patients eligible for further analysis. Moreover, all liver biopsies were scored by a single pathologist using the updated NASH CRN scoring system<sup>[6]</sup>. The pathologist was blinded to the clinical and laboratory data of the patients. The clinical, laboratory and histological data were all computerized and stored securely. The indication for liver biopsy as elicited in previous study<sup>[13]</sup> among the 238 patients with NAFLD was abnormal liver function tests followed by abnormal intraoperative appearance of liver and subsequently followed by abnormal imaging and associated abdominal pain respectively. The reasons for ineligibility are also demonstrated clearly in the original study<sup>[13]</sup>. This study was originally reviewed and approved by the institutional review board of the University of Chicago Medical Center. The informed consent was waived as part of the previous study<sup>[13]</sup> and there were no patient identifiers in the current study.

### Case definitions and liver histology

NAFLD was defined histologically by the presence of minimum 5% of steatosis on liver biopsy. Steatosis was scored as 1, 2, or 3 for 5%-33%, 34%-66%, and > 66% steatosis, respectively. Fibrosis was scored as 0, 1, 2, 3 and 4 for no fibrosis, perisinusoidal or periportal,

perisinusoidal and portal/periportal, bridging, and cirrhosis, respectively. Lobular inflammation was scored as 0, 1, 2, or 3 based on presence of no inflammation, < 2 foci per 200  $\times$  field, 2-4 foci per 200  $\times$  field, and > 4 foci per 200  $\times$  field, respectively. Ballooning was scored as 0, 1, and 2 for no balloon cell, few balloon cells, and many cells/prominent ballooning cells, respectively. Lastly, Mallory's hyaline was scored as 0 for "none to rare" or 1 for "many". A NAFLD activity score (NAS) of  $\geq 5$  was considered NASH while fibrosis score of  $\geq 2$  was considered advanced fibrosis<sup>[6]</sup>.

### Statistical analysis

Patients with NAFLD were sub-divided according to presence or absence of T2DM. Background data for NASH and NAFLD was analyzed separately. Results are expressed as mean  $\pm$  SD for continuous variables and as frequencies for categorical variables. A *t*-test for unequal variance was performed to compare the means of continuous variables. Categorical variables were compared by  $\chi^2$  test. Separate logistic regression analyses were performed to study the variables associated with presence of NASH and fibrosis. Variables which were significant on univariate analysis were included in the multivariate analysis and independent variables with *P* > 0.1 were excluded sequentially from the models. The odds ratios and associated *P*-values of the remaining variables are reported. Two-sided *P* values < 0.05 were considered statistically significant. Data analyses were performed using Stata (StataCorp, College Station, TX). The statistical methods of this study were reviewed by Matt Briggs, consultant statistician at New York Methodist Hospital.

## RESULTS

### Demographic, comorbid and biochemical features

Patients with NAFLD and T2DM, were significantly older (49.9 vs 43.0, *P* < 0.01), had higher proportion of females (71.4 vs 56.3, *P* < 0.02), and showed higher fasting glucose (167.0 vs 102.9, *P* < 0.01), higher HbA1c (8.09 vs 5.83, *P* < 0.01), higher BMI (41.0 vs 35.9, *P* < 0.01), higher international normalized ratio (1.02 vs 0.97, *P* = 0.03), higher rates of hypertension (71.4 vs 37.3, *P* < 0.01), higher rate of dyslipidemia (83.8 vs 61.2, *P* < 0.01), increased rate of metabolic syndrome (88.7 vs 36.4, *P* < 0.01) and positive indirect markers of NASH such as aspartate transaminase (AST)/alanine transaminase (ALT) ratio (0.94  $\pm$  0.4 vs 0.78  $\pm$  0.4, *P* < 0.01) and Fib-4 index (1.65 vs 1.06, *P* < 0.01) compared to patients with NAFLD without T2DM. Conversely, patients with NAFLD without T2DM had significantly higher rates of abnormal liver function tests as a leading cause of liver biopsy (74 vs 54.5, *P* = 0.02), higher ALT (79.2 vs 59.2, *P* = 0.01) and showed higher serum iron levels (89.3 vs 71.3, *P* < 0.01) compared to patients with NAFLD and T2DM (Table 1).

Background data evaluating all patients with NAFLD showed higher rate of T2DM (38.1 vs 19.4, *P* < 0.01)

**Table 1** Characteristics of patients with nonalcoholic fatty liver disease, divided according to nonalcoholic fatty liver disease without type 2 diabetes mellitus and nonalcoholic fatty liver disease with type 2 diabetes mellitus (mean  $\pm$  SD)

Parameter	All patients ( <i>n</i> = 235)	NAFLD (without T2DM) ( <i>n</i> = 158)	NAFLD (with T2DM) ( <i>n</i> = 77)	<i>P</i> value
Age (yr)	45.3 $\pm$ 11.9	43.0 $\pm$ 12.2	49.9 $\pm$ 9.8	< 0.01
Gender (male/female)	91/144	69/89	22/55	0.02
Race (% Caucasian)	65.1	63.9	67.5	0.9
BMI (kg/m <sup>2</sup> )	37.6 $\pm$ 11.5	35.9 $\pm$ 10.6	41.0 $\pm$ 12.6	< 0.01
Hypertension, <i>n</i> (%)	114 (48.1)	59 (37.3)	55 (71.4)	< 0.01
Dyslipidemia, <i>n</i> (%)	139 (68.8)	82 (61.2)	57 (83.8)	< 0.01
Metabolic syndrome, <i>n</i> (%)	115 (53.7)	52 (36.4)	63 (88.7)	< 0.01
Abnormal LFT's as an indication of liver biopsy, <i>n</i> (%)	159 (67.6)	117 (74.0)	42 (54.5)	0.02
Platelets	257.5 $\pm$ 73.5	262.6 $\pm$ 67.7	247.4 $\pm$ 83.3	0.15
INR	0.99 $\pm$ 0.16	0.97 $\pm$ 0.10	1.02 $\pm$ 0.23	0.03
Protein (g/dL)	7.4 $\pm$ 0.72	7.4 $\pm$ 0.73	7.3 $\pm$ 0.70	0.12
Albumin (g/dL)	4.3 $\pm$ 0.5	4.3 $\pm$ 0.5	4.2 $\pm$ 0.46	0.06
AST (U/L)	49.8 $\pm$ 34.5	50.4 $\pm$ 38.4	48.3 $\pm$ 24.8	0.6
ALT (U/L)	72.6 $\pm$ 58.5	79.2 $\pm$ 65.4	59.2 $\pm$ 38	0.01
Bilirubin (mg/dL)	0.67 $\pm$ 0.4	0.68 $\pm$ 0.4	0.62 $\pm$ 0.3	0.3
Alkaline Phosphate	96.7 $\pm$ 61.8	94.9 $\pm$ 68.2	100.1 $\pm$ 46.9	0.5
Total cholesterol (mg/dL)	200.2 $\pm$ 47.3	200.9 $\pm$ 45.4	198.8 $\pm$ 50.9	0.8
HDL cholesterol (mg/dL)	44.3 $\pm$ 11.8	44.2 $\pm$ 12.0	44.6 $\pm$ 11.7	0.9
Triglycerides (mg/dL)	211.4 $\pm$ 119.9	209.7 $\pm$ 127.7	214.4 $\pm$ 105.9	0.8
LDL cholesterol (mg/dL)	118.9 $\pm$ 41.0	120.5 $\pm$ 41.3	116.4 $\pm$ 40.8	0.6
Fasting glucose (mg/dL)	125.6 $\pm$ 55.5	102.9 $\pm$ 28.3	167.0 $\pm$ 67.8	< 0.01
HbA1c	6.93 $\pm$ 2.09	5.83 $\pm$ 1.11	8.09 $\pm$ 2.26	< 0.01
Iron ( $\mu$ g/dL)	82.6 $\pm$ 38.7	89.3 $\pm$ 40.8	71.3 $\pm$ 32.0	< 0.01
Total iron binding capacity ( $\mu$ g/dL)	322.4 $\pm$ 72.3	317.16 $\pm$ 71.8	333.0 $\pm$ 72.9	0.2
Ferritin (ng/mL)	258.2 $\pm$ 306.0	281 $\pm$ 323.1	223.0 $\pm$ 276.7	0.3
AST/ALT	0.836 $\pm$ 0.432	0.781 $\pm$ 0.434	0.947 $\pm$ 0.41	< 0.01
FIB-4 index	1.26 $\pm$ 1.01	1.06 $\pm$ 0.77	1.65 $\pm$ 1.28	< 0.01
APRI	0.581 $\pm$ 0.471	0.549 $\pm$ 0.458	0.643 $\pm$ 0.493	0.16

NAFLD: Nonalcoholic fatty liver disease; T2DM: Type 2 diabetes mellitus; AST: Aspartate transaminase; ALT: Alanine transaminase; LDL: Low-density lipoprotein cholesterol; NAS: NAFLD activity score; HDL: High-density lipoprotein cholesterol; BMI: Body mass index; INR: International normalized ratio; APRI: AST to platelet ratio index; LFT: Liver function test.

and presence of cirrhosis (8.3 vs 0.0,  $P = 0.01$ ) among patients with NASH compared to those with steatosis (fatty liver) alone. Moreover, there was significant higher values of AST (54.2 vs 38.3,  $P < 0.01$ ), ALT (78.7 vs 57.0,  $P = 0.01$ ) and Bilirubin (0.71 vs 0.56,  $P = 0.01$ ) and lower values of high density lipoprotein (HDL) (42.7 vs 48.1,  $P = 0.01$ ) and platelet count (247 vs 283,  $P < 0.01$ ) among patients with NASH respectively. There was no difference in age, sex, ethnicity, BMI and various other risk factor variables between the groups (Table 2).

### Histological features

Patients with NAFLD and T2DM had significantly higher mean NAS (3.5 vs 3.0,  $P = 0.03$ ) and fibrosis scores (1.2 vs 0.52,  $P < 0.01$ ) compared to patients with NAFLD without T2DM. In addition, a significantly higher percentage of patients with NAFLD and T2DM showed advanced fibrosis (27.3 vs 13.3,  $P < 0.01$ ) and prominent ballooning (27.3 vs 13.3,  $P < 0.01$ ) compared to patients with NAFLD without T2DM. However, there were no statistically significant differences between groups regarding NAS  $\geq 5$  (29.9 vs 20.9,  $P = 0.12$ ), steatosis  $\geq 2$  (66.2 vs 55.1,  $P = 0.1$ ) and inflammation  $\geq 2$  (9.1 vs 6.3,  $P = 0.4$ ). On the contrary, there was a trend for presence of higher Mallory bodies in patients with NAFLD and T2DM compared to patients with

NAFLD without T2DM (28.6 vs 17.7,  $P = 0.06$ ) (Table 3).

### NASH

After controlling for age, gender, ethnicity and BMI, a multivariate analysis showed significant association between NASH and presence of diabetes (OR = 3.27, 95%CI: 1.43-7.50,  $P < 0.01$ ) and ALT  $\geq 36$  IU (OR = 3.88, 95%CI: 1.15-13.1,  $P = 0.029$ ) in all patients with NAFLD, while only ALT  $\geq 36$  IU (OR = 4.21, 95%CI: 1.13-15.6,  $P = 0.03$ ) was significantly associated with NASH in patients with NAFLD and T2DM (Table 4).

### Fibrosis

After controlling for age, gender, ethnicity and BMI on multivariate analysis, only T2DM (OR = 3.45, 95%CI: 1.53-7.77,  $P < 0.01$ ) and low platelet (OR = 0.99, 95%CI: 0.99-1.0,  $P = 0.025$ ) showed an independent association for advanced fibrosis among all patients with NAFLD. No separate indicator for advanced fibrosis was noted among patients with NAFLD and T2DM (Table 5).

## DISCUSSION

Research over the past few decades has dwelled upon identifying the potential risk factors associated with NASH and advanced fibrosis which indeed has

**Table 2 Characteristics of patients with nonalcoholic fatty liver disease, divided according to nonalcoholic steatohepatitis and steatosis (mean + SD)**

Parameter	All patients (n = 235)	NASH	Steatosis	P value
Age (yr)	45.3 + 11.9	45.9 + 12.4	43.8 + 10.4	0.2
Gender (male/female)	91/144	67/101	24/43	0.6
Race (% caucasian)	65.1	64.9	65.7	0.1
BMI (kg/m <sup>2</sup> )	37.7 + 11.5	37 + 10.6	39.4 + 13.7	0.17
Hypertension, n (%)	114 (48.5)	82 + 48.8	32 + 63.9	1
Dyslipidemia, n (%)	139 (68.8)	100 (70.9)	39 (63.9)	0.3
Metabolic syndrome, n (%)	115 + 53.7	67 (42.7)	32 (56.1)	0.08
Platelets	258 + 73.5	247 + 72.1	283 + 71.1	< 0.01
INR	0.996 + 0.162	1 + 0.178	0.98 + 0.112	0.3
Protein (g/dL)	7.39 + 0.762	7.39 + 0.733	7.37 + 0.714	0.8
Albumin (g/dL)	4.27 + 0.536	4.27 + 0.555	4.26 + 0.487	0.8
AST (U/L)	49.8 + 34.5	54.2 + 36.3	38.3 + 26.1	< 0.01
ALT (U/L)	72.6 + 58.5	78.7 + 63.1	57 + 40.7	0.01
Bilirubin (mg/dL)	0.668 + 0.416	0.71 + 0.456	0.567 + 0.275	0.01
Alkaline Phosphate	96.7 + 61.8	100 + 70.9	88.3 + 28.3	0.1
Total cholesterol (mg/dL)	200 + 47.3	202 + 48.6	197 + 44.5	0.5
HDL cholesterol (mg/dL)	44.4 + 11.9	42.7 + 10.7	48.1 + 13.6	0.01
Triglycerides (mg/dL)	211 + 120	220 + 125	191 + 105	0.18
LDL cholesterol (mg/dL)	1119 + 41	121 + 41.6	114 + 39.8	0.33
Fasting glucose (mg/dL)	126 + 55.5	129 + 55.9	117 + 53.8	0.14
HbA1c	6.93 + 2.09	7.12 + 2.19	6.37 + 1.69	0.11
Iron (µg/dL)	82.6 + 38.7	83.8 + 40.5	78.6 + 31.9	0.52
Total iron binding capacity (µg/dL)	322 + 72.3	319 + 74.6	335 + 63.4	0.31
Ferritin (ng/mL)	258 + 306	276 + 324	201 + 232	0.21
Mallory bodies	50 + 21.3	50 + 29.8	0	< 0.01
CAD	67 + 28.9	50 + 29.9	17 + 26.2	0.63
Gastric bypass	21 + 8.94	14 + 8.33	7 + 10.4	0.61
Cirrhosis	14 + 5.96	14 + 8.33	0	0.01
Steatosis	1.92 + 0.861	2.1 + 0.83	1.46 + 0.765	< 0.01
Inflammation	0.44 + 0.62	0.625 + 0.663	0	< 0.01
Fibrosis	0.753 + 1.11	1.05 + 1.21	0	< 0.01
Ballooning	0.821 + 0.712	1.15 + 0.575	0	< 0.01
NAS score	3.18 + 1.69	3.86 + 1.45	1.46 + 0.765	< 0.01

AST: Aspartate transaminase; ALT: Alanine transaminase; HDL: High-density lipoprotein cholesterol; NAS: Nonalcoholic fatty liver disease activity score; NASH: Nonalcoholic steatohepatitis; LDL: Low-density lipoprotein cholesterol; BMI: Body mass index; INR: International normalized ratio; CAD: Coronary artery disease.

**Table 3 Histological findings of patients with nonalcoholic fatty liver disease, divided according to nonalcoholic fatty liver disease without type 2 diabetes mellitus and nonalcoholic fatty liver disease with type 2 diabetes mellitus n (%)**

Parameter	All patients (n = 235)	NAFLD (without T2DM) (n = 158)	NAFLD (with T2DM) (n = 77)	P value
NAS score (mean ± SD)	3.2 ± 1.7	3.0 ± 1.6	3.5 ± 1.7	0.03
Fibrosis score (mean ± SD)	0.75 ± 1.1	0.52 ± 0.9	1.2 ± 1.3	< 0.01
Fibrosis score				< 0.01
< 2	191 (81.3)	139 (88.0)	52 (67.5)	
≥ 2	44 (18.7)	19 (12.0)	25 (32.5)	
NAS score				0.12
< 5	179 (76.2)	125 (79.1)	54 (70.1)	
≥ 5	56 (23.8)	33 (20.9)	23 (29.9)	
NAS score				0.11
< 3	90 (38.3)	66 (41.7)	24 (31.2)	
≥ 3	145 (61.7)	92 (58.3)	53 (68.8)	
Steatosis				0.1
< 2	97 (41.3)	71 (44.9)	26 (33.8)	
≥ 2	138 (58.7)	87 (55.1)	51 (66.2)	
Ballooning				< 0.01
< 2	193 (82.1)	137 (86.7)	56 (72.7)	
2	42 (17.9)	21 (13.3)	21 (27.3)	
Inflammation				0.4
< 2	218 (92.7)	148 (93.7)	70 (90.9)	
≥ 2	17 (7.3)	10 (6.3)	7 (9.1)	
Mallory bodies	50 (21.3)	28 (17.7)	22 (28.6)	0.06

NAFLD: Nonalcoholic fatty liver disease; NAS: NAFLD activity score; T2DM: Type 2 diabetes mellitus.

**Table 4 Variables associated with nonalcoholic steatohepatitis (nonalcoholic fatty liver disease activity score  $\geq 5$ ) (multivariate analysis)**

Variables	P value	Odds ratio	95%CI
All patients			
Presence of T2DM	< 0.01	3.27	1.43-7.50
ALT $\geq 36$	0.029	3.88	1.15-13.1
Protein	0.045	1.84	1.01-3.35
Patients with NAFLD without T2DM			
Protein	0.048	3.06	1.01-9.25
Patients with NAFLD with T2DM			
ALT $\geq 36$	0.03	4.21	1.13-15.6

ALT: Alanine transaminase; NAFLD: Nonalcoholic fatty liver disease; T2DM: Type 2 diabetes mellitus.

**Table 5 Variables associated with advanced fibrosis (multivariate analysis)**

Variables	P value	Odds ratio	95%CI
All patients			
Presence of T2DM	< 0.01	3.45	1.53-7.77
Platelet	0.025	0.99	0.99-1.0
INR	< 0.01	4151	97.7-176426
Patients with NAFLD without T2DM			
Platelet	0.03	0.99	0.98-0.99
INR	0.001	16950	62.4-4607293
Patients with NAFLD with T2DM			
Platelet	0.045	0.99	0.98-0.99

NAFLD: Nonalcoholic fatty liver disease; T2DM: Type 2 diabetes mellitus; INR: International normalized ratio.

significant prognostic value. T2DM is being increasingly recognized as an important risk factor in NAFLD progression, especially with NAFLD being regarded as an extension of metabolic syndrome<sup>[2-4]</sup>. However, there is a lack of definite predictors for NASH among NAFLD patients with T2DM<sup>[14,15]</sup>, thereby highlighting the need to obtain liver histopathology for accurate determination of NASH and fibrosis. Recognizing unique histological features among NAFLD patients with T2DM would enhance our understanding of disease progression and aid development of potential remedies. Prior studies evaluating the histopathological features of NAFLD among T2DM patients utilized the Brunt system of histological evaluation<sup>[3,8,16-20]</sup> and were limited by lack of a comparative control group of patients with NAFLD without T2DM<sup>[3,16,17,20,21]</sup>. To date, only one multicenter study compared the histopathological differences among patients with NAFLD with and without T2DM<sup>[5]</sup> utilizing the NAS criteria among United States population with the limitation of potential inter-observer variability due to histology being read by multiple pathologists. Therefore, we conducted a detailed comparative histological evaluation using the NAS criteria in patients with NAFLD with and without T2DM at a single center using a single well-trained histopathologist to identify factors predicting NASH and advanced fibrosis in a multiethnic United States cohort.

Our study highlights the findings that patients with

NAFLD and T2DM have higher ballooning (27.3 vs 13.3,  $P < 0.01$ ) compared to patients with NAFLD without T2DM. Ballooning is considered to be the most important feature of steatohepatitis and correlates with features of insulin resistance very well<sup>[7]</sup>. Recently, Leite *et al.*<sup>[21]</sup> examined the histological features of NAFLD in T2DM patients using the updated NASH CRN grading system and also assessed for interpathologist's agreement on histological features. Although they demonstrated presence of significant higher ballooning (42%-55%) among patients with NAFLD and T2DM, they had several limitations. The biopsies were evaluated by two pathologists, and the kappa score for inter-observer agreement for assessment of the degree of ballooning was 0.45 indicating that there was more disagreement between the pathologists than agreement. Moreover, the study sample in Leite *et al.*<sup>[21]</sup> lacked a comparative control group of patients with NAFLD without T2DM. Thus, our study overcomes the limitations of the Leite *et al.*<sup>[21]</sup> study, but also demonstrates that patients with NAFLD and T2DM have higher rates of prominent ballooning compared to patients with NAFLD without T2DM using a larger and more diverse patient population including Asians, Hispanics, African Americans and Caucasians. Interestingly, in a large clinical trial involving 173 biopsy proven pediatric NAFLD patients, Lavine *et al.*<sup>[22]</sup> demonstrated a significant improvement in hepatocyte ballooning at 96 wk of therapy with vitamin E -0.5 (95%CI: -0.8 to -0.3,  $P = 0.006$ ) and metformin -0.3 (95%CI: -0.6 to -0.0,  $P = 0.04$ ) compared to placebo. No other histological features of NAFLD had shown any significant improvement otherwise. Furthermore, Chen *et al.*<sup>[23]</sup> in a large case-control study demonstrated that diabetes is associated with higher risk of HCC (OR = 2.29, 95%CI: 2.25-2.35,  $P < 0.001$ ) and use of metformin resulted in 7% reduction in the risk of HCC in diabetic patients incrementally (adjusted OR = 0.93, 95%CI: 0.91-0.94,  $P < 0.0001$ ) by inhibiting hepatoma proliferation and inducing cell cycle arrest. Thus, hepatocyte ballooning is not only a distinct histological feature of NASH but may play an essential role in the management and prognosis of NASH. Further studies are needed to explore the exact role of hepatocyte ballooning in the progression onto cirrhosis and HCC.

While ballooning is one component of the overall NAS, our results showed that the mean NAS was significantly higher among patients with NAFLD and T2DM (3.5 vs 3.0,  $P = 0.03$ ) compared to patients with NAFLD without T2DM. However, there was no significant difference in NAS  $\geq 5$  (20.9 vs 29.9,  $P = 0.12$ ) between the two groups in our study (Table 2) which could likely be attributed to relatively smaller sample size of the patients with NAFLD and T2DM ( $n = 77$ ). NAS is different from Brunt scoring system, as it includes the active and potentially reversible features of NAFLD, such as steatosis, inflammation and ballooning, and is separate from the potentially less reversible features like fibrosis<sup>[6]</sup>. Moreover, the numeric value of the composite

NAS is distinct from the qualitative histopathologic diagnosis utilized by Brunt scoring system (*i.e.*, definite NASH, kappa score = 0.57)<sup>[7]</sup>. Furthermore, Miyaaki *et al.*<sup>[20]</sup> and Adams *et al.*<sup>[24]</sup> have pointed that patients with NAFLD and T2DM tend to have advanced fibrosis and less steatosis, which could be attributed to the natural progression of disease or advanced age of the population with T2DM at the time of evaluation. Thus variation among individual histological features of NASH among patients with NAFLD and T2DM could lower the overall composite NAS. Therefore, it is important to quantify individual histological features separately along with NAS while interpreting liver biopsies among NAFLD patients. Further large population-based studies are needed to explore the additional utility of NAS in patients with NAFLD and T2DM.

In our study, patients with NAFLD and T2DM demonstrated significantly higher mean fibrosis score and advanced fibrosis compared to patients with NAFLD without T2DM which corroborates with prior studies<sup>[5,8,20]</sup>. On adjusting for age, gender, BMI and ethnicity, T2DM was noted to be an independent predictor for NASH and advanced fibrosis among all patients with NAFLD in our study which is not surprising. Recently, Loomba *et al.*<sup>[5]</sup> conducted a cross-sectional analysis of 1069 patients with NAFLD from the NAFLD Database study and PIVENS trial to examine the association of personal and family history of T2DM to histological features of NASH and advanced fibrosis. On sub-analysis using a comparative group of patients with NAFLD without T2DM and adjusting for age, sex, ethnicity and BMI, patients with NAFLD and T2DM had increased risk of NASH (OR = 2.48, 95%CI: 1.31-4.72,  $P = 0.01$ ), any fibrosis (OR = 2.94, 95%CI: 1.49-5.81,  $P < 0.01$ ) and advanced fibrosis (OR = 6.03, 95%CI: 3.16-11.52,  $P < 0.0001$ ) which is similar to our study results. However, the diagnosis of NASH was based on modified Brunt criteria as opposed to composite NAS criteria in our study and comparative histological analysis for differences in ballooning or NAS  $\geq 5$  was lacking. Younossi *et al.*<sup>[8]</sup>, in a cohort study of 132 patients with NAFLD, demonstrated that patients with NAFLD and T2DM ( $n = 44$ ) had significantly higher rates of cirrhosis (25 vs 10.2,  $P = 0.04$ ), liver related mortality (RR = 22.83, 95%CI: 2.97-175.03,  $P = 0.003$ ) and overall mortality (RR = 3.30, 95%CI: 1.76-6.18,  $P = 0.002$ ), compared to patients with NAFLD without T2DM ( $n = 88$ ). However, there was no statistical difference for fibrosis  $\geq 2$  between the two groups (17 vs 32,  $P = 0.07$ ) in their study, which is contrary to our study and could be related to their smaller sample size.

Early diagnosis of NASH and advanced fibrosis in patients with NAFLD and T2DM has important clinical significance. In a long term follow up study of biopsy proven NAFLD patients, Ekstedt *et al.*<sup>[25]</sup> reported higher mortality particularly among patients with NASH compared to reference population with respect to liver-related causes (2.8 vs 0.2,  $P = 0.04$ ) and from cardiovascular disease (15.5 vs 7.5,  $P = 0.04$ ). Younossi *et al.*<sup>[8]</sup> in their study reported higher occurrence of cirrhosis (25 vs

10.2,  $P = 0.04$ ), overall mortality (RR = 3.30, 95%CI: 1.76-6.18,  $P = 0.002$ ) and mortality related to liver disease (RR = 22.83, 95%CI: 2.97-175.03,  $P = 0.003$ ) among diabetic patients with NAFLD as opposed to non-diabetic patients with NAFLD. Moreover, Welzel *et al.*<sup>[26]</sup>, found a 2.9 fold risk for development of HCC among diabetic patients in a recent analysis within the SEER-database. Thus, it is imperative to identify and evaluate patients with NAFLD and T2DM early on for presence of NASH and advanced fibrosis. Currently, no single non-invasive panel has been proven to be a valid substitute for a liver biopsy<sup>[27]</sup>. In our study, the indirect markers for NASH such as AST/ALT ratio ( $0.94 \pm 0.4$  vs  $0.78 \pm 0.4$ ,  $P < 0.01$ ) and Fib-4 index (1.65 vs 1.06,  $P < 0.01$ ) showed higher significance among patients with NAFLD with T2DM compared to patients without T2DM while APRI score ( $0.64$  vs  $0.54$ ,  $P = 0.16$ ) did not. The utility of most non-invasive tests is limited as a screening test to exclude advanced fibrosis due to high negative predictive value (76.9%-90.5%) and a modest positive predictive value (36.1%-61.1%)<sup>[28]</sup>. Moreover, patients with NAFLD and BMI  $> 30$  (OR = 8.4, 95%CI: 6.6-10.8,  $P < 0.0001$ ) and T2DM (OR = 2.0, 95%CI: 1.5-2.6,  $P < 0.0001$ ) pose unique challenge for accurate liver stiffness measurement using Fibroscan, mainly due to attenuation of elastic waves by subcutaneous and pre-hepatic fat thickness<sup>[29]</sup>. Therefore, a selective liver biopsy could be helpful among obese, elderly patients especially with NAFLD and T2DM to accurately stage NAFLD. Furthermore, our results (Table 5) comparing the background features among patients with NASH and steatosis showed higher number of patients with NASH to have T2DM and cirrhosis in addition to indirect evidence for advanced disease and fibrosis reflected by significantly higher Bilirubin, AST and ALT and lower platelet and lower HDL values. Therefore optimization of risk factors of NAFLD such as metabolic syndrome, T2DM and dyslipidemia to prevent further progression of liver disease to cirrhosis, HCC and other related complications is warranted.

Our study has some limitations. Data on duration of T2DM and insulin resistance were lacking, hence we could not assess for the association of these variables with NAFLD progression. However, the mean HbA1c among patients with NAFLD and T2DM was 8.09 which reflects uncontrolled T2DM thereby minimizing the confounding effect of diabetic medication and highlighting the role of uncontrolled T2DM in NAFLD progression. Furthermore, the data being obtained at a major tertiary care referral center, could have led to selection bias. Lastly, the smaller sample size of patients with NAFLD and T2DM limited our ability to show statistically significant rate of cirrhosis. Therefore, larger prospective studies on patients with NAFLD with and without T2DM are needed to further our understanding of the relationship between T2DM and NAFLD progression.

In conclusion, patients with NAFLD and T2DM tend to have higher ballooning and advanced fibrosis compared to patients with NAFLD without T2DM. Our study also demonstrated that T2DM is an independent predictor

for both NASH and advanced fibrosis, while utilizing the more current NAS criteria. Patients with NAFLD and T2DM in general should be advised and educated regarding optimal diabetes control, hyperlipidemia management and vascular disease screening which may not only prevent cardiovascular complications but also could prevent further progression to NASH and/or advanced fibrosis/cirrhosis. Future prospective studies are needed to explore the role of NASH (especially hepatocyte ballooning) among patients with and without T2DM and development of advanced fibrosis, HCC and cardiovascular disease burden and mortality.

## COMMENTS

### Background

Identifying the unique histopathological features of nonalcoholic fatty liver disease (NAFLD) among patients with type 2 diabetes mellitus (T2DM) is not only important to stage the disease progression but also help us understand the impact of diabetes in progression of NAFLD. Presently, only few studies have described the histopathological differences among NAFLD patients with and without T2DM in a multiethnic United States population cohort.

### Research frontiers

While screening for diabetes and other risk factors for NAFLD is easily performed, management of NAFLD is still evolving. Current interest is in understanding the pathogenesis of NAFLD progression, which will pave way to potential novel treatments.

### Innovations and breakthroughs

The authors' study highlights the findings that patients with NAFLD and T2DM have higher ballooning compared to patients with NAFLD without T2DM. Ballooning is considered to be the most important feature of steatohepatitis and correlates with features of insulin resistance very well. Similarly, significantly higher mean fibrosis score and advanced fibrosis is demonstrated among patients with NAFLD and T2DM. It is important to quantify individual histological features separately along with NAS while interpreting liver biopsies among NAFLD patients.

### Applications

T2DM is an independent predictor for both NASH and advanced fibrosis. Hepatocyte ballooning is not only a distinct histological feature of NASH but may play an essential role in the management and prognosis of NASH. Patients with NAFLD and T2DM in general should be advised and educated regarding optimal diabetes control, hyperlipidemia management and vascular disease screening thereby preventing progression of liver disease burden and mortality.

### Terminology

NAFLD includes a histological spectrum of liver diseases ranging from simple steatosis to NASH and the latter histological entity can progress to cirrhosis. T2DM is shown to be an independent risk factor for the development of NASH. The components that make up NASH include fat in liver cells (steatosis), inflammation, scar tissue (fibrosis) and degeneration of liver cell (ballooning).

### Peer-review

This is an excellent work dealing with a very interesting topic, the histopathological alterations in diabetic and non-diabetic patients with NAFLD. There are few studies describing the mentioned differences and this work constitutes a novel approach. The authors showed clearly the impact of T2DM on NAFLD. Comorbidity seems to be very important in converting the disease and determining the severity of the disease either ways.

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