Nonalcoholic Fatty Liver Disease in Patients With Type 2 Diabetes

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Background & Aims: **Nonalcoholic fatty liver disease (NAFLD) is reported commonly in patients with type 2 diabetes mellitus (DM), which has been suggested as a risk factor for the progressive form of NAFLD, or nonalcoholic steatohepatitis. The aim of this study was to assess the outcome of patients with NAFLD and DM.** ***Methods:*** **A cohort of patients with NAFLD was identified, and patients with other causes of liver disease (alcohol, medication, etc.) were excluded. Clinical, pathological, and mortality data were available for this cohort. Patients were categorized and compared according to the presence or absence of DM.** ***Results:*** **Of 132 patients with NAFLD, 44 patients (33%) had an established diagnosis of DM. Patients with DM were older and had greater serum glucose and triglyceride levels and a greater aspartate aminotransferase–alanine aminotransferase ratio. Liver biopsy specimens from patients with DM showed more vacuolated nuclei and acidophilic bodies. Cirrhosis (histological or clinical) occurred in 25% of patients with DM (11 of 44 patients) and NAFLD compared with only 10.2% (9 of 88 patients) of patients without DM with NAFLD ($P = 0.04$). After adjusting for potential confounders (age, body mass index, and the presence of cirrhosis), both overall mortality (risk ratio [RR], 3.30; 95% confidence interval [CI], 1.76–6.18; $P = 0.002$) and mortality related to liver disease (RR, 22.83; 95% CI, 2.97–175.03; $P = 0.003$) were greater in diabetic patients with NAFLD. Markers of hepatic dysfunction (low albumin level, high total bilirubin level, and prolonged prothrombin time) were the only independent predictors of increased mortality.** ***Conclusions:*** **Patients with NAFLD and DM are at risk for the development of an aggressive outcome, such as cirrhosis and mortality. This study supports the potential role of insulin resistance in the development of poor clinical outcomes in patients with NAFLD.**

Nonalcoholic fatty liver disease (NAFLD) is a spectrum of clinicopathologic conditions characterized by lipid deposition in liver parenchyma of patients who have no history of excessive alcohol use. Within this spectrum, steatosis alone is apparently benign, but nonalcoholic steatohepatitis (NASH), characterized by ballooning degeneration and sinusoidal/pericellular fibrosis, can be progressive.[1–10] Increasing evidence suggests that patients with diabetes mellitus (DM) are particularly at risk for developing the progressive form of NAFLD, i.e., NASH.[10–12] Progression in diabetic patients with NAFLD may be related to a number of pathophysiologic mechanisms associated with insulin resistance.[1,2,13–25] This study uses an existing NAFLD database to assess long-term outcomes of patients with DM and NAFLD and compare them with outcomes of patients without DM with NAFLD.

Patients and Methods

Development of the NAFLD Database

The NAFLD database was created by looking at liver biopsies processed at the Cleveland Clinic Department of Pathology (Cleveland, OH) from January 1, 1979, to December 31, 1987. Pathological features identified excess fat with or without other pathological findings. Specimens with other causes of liver disease (e.g., alcohol, medication, hepatitis C, iron overload) were systematically excluded.[3,4,26] Data included a large number of clinical and pathological features, as well as long-term mortality data (time and cause of death).[3,18] For the purpose of this analysis, patients were considered to have DM if they were clinically diagnosed with type 2 DM and receiving treatment for it (oral hypoglycemic agents, insulin, or both).

Statistical Analysis

Comparisons of continuous normal variables were made using an analysis of variance. Categorical variables were compared using the χ^2 or Fisher exact test, survival estimates were computed using the Kaplan-Meier method, and overall survival for patients with and without DM was compared using the log-rank test. Univariate logistic regression analysis was used to identify demographic and pathological features

Abbreviations used in this paper: CI, confidence interval; DM, diabetes mellitus; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; RR, risk ratio.

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Table 1. Comparison of Patients With NAFLD According to the Presence of Cirrhosis

Features	Noncirrhotics (N = 112)	Cirrhotics (N = 20)	*P*
Age at biopsy (*yr*)	51.98 ± 14.54	59.06 ± 13.89	0.05
Prothrombin time (*s*)	12.33 ± 0.95	13.83 ± 1.36	<0.01
Total bilirubin (*mg/dL*)	0.89 ± 0.97	1.48 ± 1.14	0.04
Albumin (*g/dL*)	4.14 ± 0.70	3.77 ± 0.75	0.04
Type 2 diabetes (*%*)	29 (33/112)	55 (11/20)	0.04
AST (*U/L*)	42.86 ± 43.68	71.80 ± 45.71	0.01
AST-ALT ratio	0.90 ± 0.34	1.77 ± 0.98	0.03
Grade of inflammation (≥2) on the index biopsy (*%*)	46 (52/112)	75 (15/20)	0.03
Mallory bodies on the index biopsy (*%*)	13 (15/112)	35 (7/20)	0.04
Hepatocyte necrosis on index biopsy (*%*)	36 (40/112)	75 (15/20)	<0.01

ALT, alanine aminotransferase; AST, aspartate aminotransferase; NAFLD, nonalcoholic fatty liver disease.

significantly associated with an outcome of cirrhosis in patients with DM. Odds ratios, their 95% confidence intervals (CIs), and the model *P* also were obtained. Univariate Cox proportional hazards analysis was used to identify factors associated with overall mortality and liver-related mortality. Results are summarized as the *P* of the model, adjusted risk ratios (RRs), and 95% CIs. To determine which factors were independently associated with an outcome (cirrhosis, mortality, and liver-related mortality), multivariate analysis was performed using variables with significance in the univariate analysis. For all our analyses, $P < 0.05$ is considered significant, unless otherwise noted. All analyses were performed using SAS software (SAS Institute, Cary, NC).

Results

Identification of the NAFLD Cohort

A total of 4238 liver biopsy specimens were processed at the Cleveland Clinic Department of Pathology during the period of this study. Of these, 772 specimens (18%) showed excessive fatty accumulation (± other features) as their primary diagnosis. Specimens with other causes of liver disease were excluded. Specimens from the remaining 157 patients (3.7%) fulfilled criteria for the final diagnosis of NAFLD. Of these, 132 patients (84%) had complete clinical and pathological data and constituted our NAFLD cohort (48%, men; 88%, white; 33%, DM; mean follow-up, 10 yr).

For the entire NAFLD cohort, cirrhosis (clinical or histological) occurred in 15% (20 of 132 patients), with 9 patients showing histological cirrhosis on the index biopsy specimen and evidence for clinical cirrhosis (e.g., ascites, hepatic encephalopathy, or variceal bleeding) during follow-up. Another 11 patients with NAFLD who did not have histological cirrhosis on the index biopsy specimen developed clinical cirrhosis during follow-up. Characteristics of patients with NAFLD with and without cirrhosis are listed in Table 1.

Of the NAFLD cohort, 33% (44 of 132 patients) met our criteria for DM. Patients with DM were older and had greater serum glucose and serum triglyceride levels and a greater aspartate aminotransferase–alanine aminotransferase ratio (Table 2). Furthermore, liver biopsy specimens from patients with DM showed more evidence for vacuolated nuclei and acidophilic bodies (Table 2). Although not statistically significant, diabetic patients

Table 2. Comparison of Clinical, Laboratory, and Pathological Features for Patients With NAFLD: With and Without DM

Features	DM (n = 44)	No DM (n = 88)	*P*
Age at biopsy (*yr*)	57.0 ± 10.5	51.1 ± 16.0	0.01
Body mass index (kg/m^2)	30.8 ± 5.7	28.8 ± 5.9	0.07
AST (*U/L*)	57.2 ± 60.4	42.3 ± 33.9	0.06
AST-ALT ratio	1.5 ± 0.7	0.9 ± 0.5	0.01
Prothrombin time (*s*)	13.0 ± 1.5	12.4 ± 0.9	0.02
Albumin (*g/dL*)	3.9 ± 0.7	4.2 ± 0.7	0.06
Serum glucose (*g/dL*)	172.9 ± 66.8	110.2 ± 32.3	<0.001
Serum triglyceride (*mg/dL*)	489.3 ± 374.8	226.6 ± 108.2	0.03
Gender (*% male*)	36	54	0.06
Ethnicity (*% white*)	84	90	0.08
Abdominal pain (*% present*)	59	40	0.04
Grade of fibrosis ≥2 (*% present*)	17	32	0.07
Vacuolated nuclei (*% present*)	43	17	0.01
Acidophilic bodies (*% present*)	9	0	0.01

DM, diabetes mellitus; AST, aspartate aminotransferase; ALT, alanine aminotransferase; NAFLD, nonalcoholic fatty liver disease.

Table 3. Features Associated With Cirrhosis in Patients With Diabetes With NAFLD

Features	Odds ratios (95% CI)	*P*
Prothrombin time (*1 s increase*)	5.90 (1.78–19.45)	0.004
Total bilirubin (*1 mg/dL increase*)	77.0 (4.30–99.00)	0.003
Albumin (*1 g/dL increase*)	0.10 (0.02–0.42)	0.002
Cholesterol (*1 mg/dL increase*)	0.97 (0.94–0.99)	0.01
Liver cell necrosis on the index biopsy (*present vs. absent*)	9.00 (1.65–49.00)	0.01

NAFLD, nonalcoholic fatty liver disease; CI, confidence interval.

with NAFLD tended to be women and nonwhite and have a greater body mass index, greater aspartate aminotransferase level, and higher grade of fibrosis on their index liver biopsy specimens (Table 2).

Of diabetic patients with NAFLD, 25% (11 of 44 patients) had cirrhosis (histological or clinical) compared with 10.2% (9 of 88 patients) in patients without DM ($P = 0.04$). Factors associated with cirrhosis in diabetic patients with NAFLD are listed in Table 3. The multivariate model showed that only prothrombin time (RR, 28.04; 95% CI, 1.98–396) was independently associated with cirrhosis in patients with DM and NAFLD.

Overall mortality of diabetic patients with NAFLD was 56.8% (25 of 44 patients) compared with 27.3% (24 of 88 patients) in patients without DM with NAFLD ($P = 0.001$). After adjusting for potential confounders (age, body mass index, and presence of cirrhosis), overall mortality remained greater in patients with DM and NAFLD (RR, 3.30; 95% CI, 1.76–6.18; $P = 0.002$). Factors associated with overall mortality in patients with DM and NAFLD are listed in Table 4. Multivariate analysis showed that prothrombin time (RR, 1.78; 95% CI, 1.04–3.04) and albumin level (RR, 0.23; 95% CI, 0.065–0.83) were independently associated with increased mortality.

Liver-related deaths occurred in 18.2% of diabetic patients with NAFLD (8 of 44 patients) compared with 2.3% in patients with NAFLD without DM (2 of 88 patients; $P = 0.02$). After adjusting for the same confounders, risk for liver-related mortality remained greater in patients with DM and NAFLD (RR, 22.83; 95% CI, 2.97–175.03; $P = 0.003$). Factors associated with liver-related mortality are listed in Table 5. Of these factors, only total bilirubin level (RR, 3.0; 95% CI, 1.31–6.87) remained independently associated with liver-related mortality.

Table 4. Features Associated With Mortality in Patients With Diabetes and NAFLD

Features	Relative risks (95% CI)	*P*
Prothrombin time (*1 s increase*)	1.35 (1.03–1.78)	0.033
Total bilirubin (*1 mg/dL increase*)	2.12 (1.34–3.36)	0.001
Albumin (*1 g/dL increase*)	0.21 (0.09–0.53)	<0.001
Grade of fibrosis ≥2 on the index biopsy (*present*)	2.92 (1.27–6.71)	0.012

CI, confidence interval; NAFLD, nonalcoholic fatty liver disease.

Table 5. Features Associated With Liver-Related Mortality in Patients With Diabetes and NAFLD

Features	Relative risks (95% CI)	*P*
Prothrombin time (*1 s increase*)	1.89 (1.24–2.87)	0.003
Total bilirubin (*1 mg/dL increase*)	3.37 (1.67–6.77)	0.0007
Albumin (*1 g/dL increase*)	0.078 (0.02–0.398)	0.002
Cholesterol (*1 mg/dL increase*)	0.975 (0.95–0.996)	0.022

CI, confidence interval; NAFLD, nonalcoholic fatty liver disease.

Discussion

This analysis indicates that patients with DM and NAFLD have more aggressive disease with respect to cirrhosis and mortality than NAFLD patients without DM. The increased risk remained significant even after adjusting for potentially important confounders that can affect survival. The 18.2% liver-related mortality rate reported here is much greater than that of patients without DM with NAFLD and those reported for the general population.[3,4] Although a number of factors were associated with cirrhosis, mortality, or liver-related mortality, only those reflecting hepatic dysfunction (low albumin level, coagulopathy, high total bilirubin level) were independently associated with these long-term outcomes. Furthermore, patients with NAFLD and a clinically established diagnosis of DM had evidence of other conditions associated with metabolic syndrome (obesity, hyperglycemia, and hyperlipidemia). This finding is not surprising considering that DM is a manifestation of metabolic syndrome, strongly associated with NAFLD.

Additionally, this analysis shows that patients with NAFLD and cirrhosis more commonly had DM and a greater prevalence of pathological features consistent with the diagnosis of NASH (such as hepatocyte necrosis, Mallory bodies, higher grades of inflammation, and fibrosis).

The origin of worse histological and clinical outcomes in patients with NAFLD and DM remains unclear. However, DM increasingly has been associated with chronic inflammation,[18,19] oxidative stress,[20–23] and the up-regulation of hepatotoxic cytokines,[24,25] all mechanisms implicated in the pathophysiological state of NAFLD.

The most important shortcoming of this study is the selection bias associated with the inclusion of patients from a tertiary-care center. In addition, the retrospective nature of data collection did not allow important laboratory assays for the assessment of metabolic syndrome (e.g., serum insulin level). This bias may result in an underestimate of the true prevalence of insulin resistance, rather than clinically overt DM, in patients with NAFLD. Nevertheless, the in-depth design and long-term outcomes collected for this study provide a unique contribution to the literature relating aggressive liver disease to the combination of NAFLD and DM.

In summary, our data indicate that patients with NAFLD and DM experience greater rates of cirrhosis and mortality. This has important clinical and prognostic implications for patients with NAFLD. Patients with clinical evidence of NAFLD and DM may have more progressive liver disease. Such patients should be the target of future investigations into the pathogenesis of NAFLD and NASH and clinical trials designed for the treatment of NASH.[1,2,4,10,26,27]

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