HEPATOLOGY
Official Journal of the American Association for the Study of Liver Diseases

Prevalence and Natural History of Histologically Proven Chronic Liver Disease in a Longitudinal Cohort of Patients With Type 1 Diabetes

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Although a higher prevalence of raised liver enzymes and altered echotexture on ultrasound have been reported in patients with type 1 diabetes mellitus (T1DM), the histological spectrum and natural history of chronic liver disease (CLD) in T1DM is unknown. We investigated the prevalence and outcome of histologically proven CLD in a longitudinal cohort of patients with T1DM. We identified patients who have had liver biopsy from a computerized database (DIAMOND; Hicom Technology, Brookwood, UK) containing longitudinal data for over 95% of type 1 diabetes patients from an overall catchment population of 700,000 people. Gender-matched patients with oral hypoglycemic-treated (T2OH) and insulin-treated type 2 diabetes (T2IN) who had liver biopsy formed two comparative cohorts. We collated clinical and histological data, as well as long-term outcomes of all three groups, and compared T1DM cirrhosis incidence to UK general population data. Of 4,644 patients with T1DM, 57 (1.2%) underwent liver biopsy. Of these, 53.1% of patients had steatosis, 20.4% had nonalcoholic steatohepatitis, and 73.5% had fibrosis on index liver biopsy. Cirrhosis was diagnosed in 14 patients (24.6%) during follow-up. T1DM with age under 55 years had an odds ratio of 1.875 (95% confidence interval: 0.936-3.757) for cirrhosis incidence, compared to the general population. Longitudinal liver-related outcomes were similar comparing the T1DM cohort and respective type 2 diabetes cohorts—when adjusted for important confounders, diabetic cohort type did not predict altered risk of incident cirrhosis or portal hypertension. *Conclusion:* Type 1 diabetes is associated with a previously unrecognized burden of CLD and its complications. (HEPATOLOGY 2014;60:158-168)

Type 2 diabetes and nonalcoholic fatty liver disease (NAFLD) are intimately related, with insulin resistance (IR) and subsequent hyperinsulinaemia being critical steps for their pathogenesis.[1] Type 2 diabetes is a well-recognized risk factor for the development and progression of NAFLD. The estimated prevalence of type 2 diabetes in Western populations has continued to rise in recent decades[2,3] and is approximately 4.5% in the UK[4]; this increases to 18%-45% in patients with NAFLD.[5] Confirming this association, the prevalence of NAFLD rises from an estimated 34% in the general population[6] to up to 70% in patients with type 2 diabetes.[7] Patients with type 2 diabetes show an increased prevalence of nonalcoholic steatohepatitis[8,9] (NASH; the

Abbreviations: ALT, alanine aminotransferase; BMI, body mass index; CI, confidence interval; CLD, chronic liver disease; CTGF, connective tissue growth factor; GPRD, General Practice Research Database; HCC, hepatocellular carcinoma; HR, hazard ratio; IR, insulin resistance; IRR, incidence rate ratio; MetS, metabolic syndrome; MODY, maturity onset diabetes of the young; NAFLD, nonalcoholic fatty liver disease; NAS, NAFLD activity score; NASH, nonalcoholic steatohepatitis; NUH, Nottingham University Hospitals; OR, odds ratio; PH, portal hypertension; T1DM, type 1 diabetes mellitus; T2OH, type 2 diabetes mellitus requiring dietary change or oral hypoglycemic agents; T2IN, type 2 diabetes requiring insulin; TG, triglyceride; US, ultrasound.

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Received September 13, 2013; accepted February 25, 2014.

Supported by the National Institute for Health Research (NIHR) Nottingham Digestive Diseases Biomedical Research Unit based at Nottingham University Hospitals NHS Trust and University of Nottingham. The views expressed are those of the author(s) and not necessarily those of the National Health Service, the NIHR, or the Department of Health.

progressive subtype of NAFLD), advanced liver fibrosis and cirrhosis,[10-12] hepatocellular carcinoma (HCC),[12,13] as well as liver-related mortality.[14-16]

In contrast, type 1 diabetes has not been regarded as a significant risk factor for chronic liver disease (CLD). Small case series have reported on the presence of hepatic glycogenosis, a benign, readily reversible condition resulting from persistent hyperglycemia in patients with type 1 diabetes.[17,18] However, recent studies demonstrated an increased prevalence of elevated alanine aminotransferase (ALT) levels in patients with type 1 diabetes,[19,20] one that was higher than expected in the general population and comparable with patients with type 2 diabetes. Using ultrasonographic features, 44% of a group of patients with type 1 diabetes were considered to have NAFLD.[21]

However, neither ALT elevation nor ultrasound (US) features are specific for the diagnosis of CLD. We have investigated the prevalence and outcome of histologically proven CLD in a longitudinal cohort of patients with type 1 diabetes.

Patients and Methods

Study Site and Databases. Nottingham University Hospitals (NUH) has a potential catchment population of 700,000 patients and serves as a regional tertiary referral unit for both hepatology and diabetology. Over 95% of the catchment population's patients with type 1 diabetes, and approximately 25% of patients with type 2 diabetes, attend the clinic in secondary care. Clinical information for these patients is prospectively collected on a computerized database (DIAMOND; Hicom Technology, Brookwood, UK). Collated data include relevant clinical information, anthropometric measurements (including weight, height, and body mass index [BMI] calculation), and standard hematology and biochemistry results taken as part of standard care. Longitudinal patient data are collected until the point of discharge from clinic or death, whichever occurs first. A second database (Trent Pathology System, McKesson until 2008; Winpath, MSC 2008 to present) prospectively collects information for all histological analyses performed at NUH since 1991.

Study Design. The DIAMOND and histopathology databases were cross-matched to establish patients with type 1 or 2 diabetes undergoing liver biopsy at our unit between January 1991 and December 2011. Patients with type 1 diabetes formed the type 1 diabetes mellitus (T1DM) cohort. For each patient with type 1 diabetes who underwent liver biopsy, we identified 1 gender-matched non-insulin-treated (T2OH cohort) and one insulin-treated type 2 diabetes patient (T2IN cohort) who had undergone liver biopsy. Both type 2 diabetes cohorts were defined by diabetic treatment at the time of index liver biopsy and populated by use of a random number generator from the entire type 2 diabetes population undergoing liver biopsy. Patients with gestational diabetes, maturity-onset diabetes of the young (MODY), secondary diabetes, or liver biopsy performed before diabetes diagnosis were excluded from the study.

Indications for liver biopsy as well as histological and clinical diagnoses established at index liver biopsy were identified for all included patients. We analyzed clinical data from the time of index liver biopsy, including anthropometric measures, laboratory parameters (full blood count, creatinine, and liver enzymes) and markers of parenchymal function (bilirubin, albumin, and coagulation studies), and medical history (including prevalent cardiovascular disease, malignancy, and microvascular complications of diabetes). Hypertension was defined as the use of one or more antihypertensive agents or blood pressure persistently recorded as greater than 140/90 mmHg. Hyperlipidemia was defined as the use of one or more lipid-lowering agents or triglyceride (TG) levels greater than 1.7 mmol/L. Because waist or hip circumference was not measured for most patients, a BMI greater than 30 kg/m^2 defined obesity. Metabolic syndrome (MetS) was defined as per World Health Organization criteria.[22] The date of each patient's diabetes diagnosis was also recorded.

Histology. Liver biopsy specimens were routinely stained with hematoxylin and eosin, picrosirius red stain, and Masson's trichrome for connective tissue assessment as well as Perl's stain for hepatic iron quantification. NAFLD activity score (NAS) scores were assessed using the criteria proposed by Kleiner et al.[23]; steatosis was graded on a 4-point scale: grade 0 = steatosis involving <5% of hepatocytes; grade 1 = 5%-33%; grade 2 = 33%-66%; and grade

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DOI 10.1002/hep.27098

Potential conflict of interest: Nothing to report.

3 = >66%. Lobular inflammation was graded on a 4-point scale: grade 0 = no foci of inflammation; grade 1 = <2 foci per 200× field; grade 2 = 2-4 foci; and grade 3 = >4 foci. Hepatocyte ballooning was graded on a 3-point scale: grade 0 = no evidence of ballooning; grade 1 = few ballooning cells; and grade 2 = prominent ballooning. Fibrosis was graded both on a 5-point scale: grade 0 = none; grade 1 = perisinusoidal or periportal fibrosis; grade 2 = perisinusoidal and portal/periportal fibrosis; grade 3 = bridging fibrosis; and grade 4 = cirrhosis, and as a percentage. Additionally, nuclear and cytoplasmic glycogenosis and hepatic iron were scored on a 4-point scale, and pericellular fibrosis and portal inflammation were scored as previously described by Brunt et al.[24]

For comparison of histological staging and activity between the T1DM and respective type 2 diabetes cohorts, liver biopsy specimens were read by an experienced consultant histopathologist (P.K.) who was blinded to cohort assignment and clinical history. Specimens from patients with metastatic malignancy (n = 26), or those patients with missing histology specimens (n = 5), were excluded from histological subanalysis.

Longitudinal Outcomes. Overall survival and incidence of longitudinal liver-related outcomes (cirrhosis, portal hypertension [PH], and HCC) were identified and compared between the T1DM and respective type 2 diabetes cohorts. Patients free of the outcomes at the time of diabetes diagnosis were followed for developing the outcomes of interest using regular follow-up clinic visits. Cumulative follow-up time from diabetes diagnosis to each of the outcomes (death, cirrhosis, PH, or HCC) was separately computed as months. Patients who did not develop any of the outcomes were censored from follow-up at the end of the study period (April 2012). Liver cirrhosis was defined by histological criteria in more than 90% of cases or established radiological and clinical criteria in cases where cirrhosis developed after index liver biopsy. PH was defined as the presence of varices at endoscopy, whereas ascites were attributed to cirrhotic PH or variceal bleeding. Furthermore, cases of incident HCC were identified from the two databases. Patients were excluded from longitudinal outcomes analysis if presenting with metastatic malignancy at liver biopsy (n = 26), if less than 1 year exposure time between diabetes diagnosis and development of the specified outcome (as not an incident event; n = 4), or where the date of diabetes diagnosis could not be established (n = 5).

Incidence of liver cirrhosis in all patients with type 1 diabetes on the DIAMOND database was subsequently compared to general population estimates. Cumulative follow-up years since diabetes diagnosis were established using the DIAMOND database. To calculate overall cirrhosis incidence in the type 1 diabetes cohort, we used the total number of new cases of cirrhosis as the numerator and the cumulative follow-up years of the entire type 1 diabetes population on the DIAMOND database as the denominator. Age-standardized incidence rate ratios were calculated comparing type 1 diabetes cirrhosis incidence to published UK General Practice Research Database (GPRD) data.[25] To allow direct comparison with GPRD data, and ensure only incident cirrhosis was captured, patients (n = 3) diagnosed with cirrhosis within the first year of their type 1 diabetes diagnosis or diagnosed with cirrhosis before the age of 25 (n = 3) were excluded from this analysis.

Statistical Analysis. Statistical analysis was performed using SPSS statistical software (version 19.0; IBM, Armonk, NY). Categorical data are presented as number (percentage). Continuous data are presented as mean (standard deviation; SD) for parametric data and medians (range) for nonparametric data. Continuous variables were compared using the two-sample *t* test for parametric variables and Mann-Whitney's test for nonparametric variables. Categorical variables were compared using the chi-squared test or Fisher's exact test, where appropriate. Statistical comparison was performed individually between the T1DM cohort and respective type 2 diabetes cohorts. A two-tailed *P* value of less than 0.05 was considered statistically significant.

Baseline histologic features were compared using Fisher's exact test between the T1DM cohort and individual type 2 diabetes cohorts: (T1DM vs. T2OH and T1DM vs. T2IN). The prevalence of steatosis (any grade), fibrosis (any stages), and advanced fibrosis (≥ stage 3) was also compared using logistic regression models with and without adjustment for potential confounders, including age at index liver biopsy and the presence of obesity. Separate models were developed for T1DM versus T2OH and T1DM versus T2IN.

Comparisons of overall survival and incidence of liver-related outcomes between the cohorts were performed using Kaplan-Meier's method. The beginning of the Kaplan-Meier curve was defined as the date of diabetes diagnosis for liver-related outcomes, and date of index liver biopsy for survival, with patients subsequently censored as described previously. The log-rank

Table 1. Baseline Characteristics of Patients at Index Liver Biopsy

Variable	T1DM Cohort (n = 57)	T2OH Cohort (n = 57)	*P* Value (T1DM vs. T2OH)	T2IN Cohort (n = 57)	*P* Value (T1DM vs. T2IN)
Age, years	43.7 (17.8)	59.7 (11.6)	**<0.001**	61.4 (10.0)	**<0.001**
Male, n (%)	32 (56.1)	32 (56.1)	1.00	32 (56.1)	1.00
Interval since diabetes diagnosis, months	119.5 (25.5-303.25)	56 (27-94)	**0.009**	141.0 (60-202)	0.778
Hypertension, n (%)	23 (40.4)	45 (78.9)	**<0.001**	44 (77.2)	**<0.001**
Hyperlipidemia, n (%)	33 (57.9)	37 (64.9)	0.586	46 (80.7)	**0.007**
BMI, kg/m^2	25.2 (5.4)	30.6 (4.7)	**<0.001**	30.2 (5.7)	**<0.001**
MetS, n (%)	10 (17.5)	24 (42.1)	**0.004**	23 (40.4)	**0.004**
Hazardous alcohol use*, n (%)	14 (24.6)	8 (14.0)	0.24	9 (15.8)	0.35
Microvascular diabetes complications, %	24 (42.1)	11 (19.3)	**0.011**	29 (50.9)	0.344
Platelet count, 10^9 /L	246.0 (114.1)	234.9 (86.4)	0.283	226.1 (100.2)	0.149
Creatinine, μmol/L	81.0 (66.5-99.5)	84.0 (69.0-99.0)	0.391	93.0 (76.0-133.5)	**0.004**
ALT, U/L	67.0 (32.0-114.0)	53.0 (39.0-89.0)	0.335	44.5 (25.3-111.8)	0.119
GGT, U/L	177.0 (70.5-418)	224.0 (96.0-349.0)	0.420	157.0 (77.0-537.5)	0.663
Bilirubin, μmol/L	13.0 (8.0-21.5)	12.0 (8.75-18.25)	0.903	12.0 (7.5-22.0)	0.858
Albumin, g/L	34.4 (7.5)	37.4 (7.2)	**0.037**	33.1 (7.35)	0.364
Prothrombin time (seconds)	10.0 (10.0-13.0)	11.0 (10.0-12.0)	0.425	12.0 (10.0-14.0)	**0.016**

Normally distributed numerical variables are displayed as mean (standard deviation) and have been compared using the two-sample *t* test. Non-normally distributed variables are displayed as median (interquartile range) and have been compared using Mann-Whitney's *U* test. Categorical variables are displayed as n (%) and have been compared using Fisher's exact test. *P* values $\leq$0.05 denote statistically significant differences and are highlighted in bold.

*Hazardous alcohol use defined as >14 units per week in female patients and >21 units per week in male patients.

Abbreviation: GGT, gamma-glutamyl transpeptidase.

test was used to calculate differences in outcome incidence between the cohorts (T1DM vs. T2OH and T1DM vs. T2IN). Cox's proportional hazard models were used to compare incidence of outcomes between the cohorts with and without adjusting for potential confounders (i.e., age of diabetes diagnosis, presence of obesity, hazardous alcohol use, and hepatitis C exposure). Separate models were developed for T1DM versus T2OH and T1DM versus T2IN.

Results

Clinical Characteristics of Study Population. We identified 4,641 patients with type 1 diabetes on the DIAMOND database, of whom 57 had undergone liver biopsy and formed the T1DM cohort. We identified 9,571 patients with type 2 diabetes on the DIAMOND database, of whom 270 underwent liver biopsy during the study period. We excluded 41 patients with type 2 diabetes, of whom 37 underwent index liver biopsy before formal diabetes diagnosis, and 4 had no medication data. From the remaining 229 patients, we randomly assigned 57 with diet or oral hypoglycaemic therapy for type 2 diabetes at the time of index biopsy (the T2OH cohort) and 57 with insulin therapy for type 2 diabetes (the T2IN cohort); all were gender matched with the T1DM cohort.

Baseline characteristics for each cohort at index biopsy are shown in Table 1. The T1DM cohort was significantly younger than either type 2 diabetes cohort (43.7 vs. 59.7 and 61.4 years; $P < 0.001$ for both). The T1DM cohort had a significantly lower prevalence of both individual features of MetS and fulfilled diagnostic criteria (MetS prevalence in the T1DM cohort was 17.5% vs. 42.1% and 40.4%; $P = 0.004$ for both). Median diabetes duration before biopsy was longer in the T1DM cohort, compared to the T2OH cohort (119.5 vs. 56.0 months; $P = 0.009$), but not significantly different from the T2IN patients.

Primary diagnoses for each cohort after index liver biopsy are shown in Table 2. Hepatic glycogenosis was the primary diagnosis in 8 patients (14%) with type 1 diabetes and 1 (1.8%) in each matched type 2 diabetes cohort ($P = 0.03$ for both). The T1DM cohort was significantly less likely to be diagnosed with NAFLD than T2OH patients (19.3% vs. 40.4%; $P = 0.02$), but not T2IN patients (19.3% vs. 28.1%; $P = 0.38$). There were no other significant differences in commonly recorded diagnoses between type 1 and 2 diabetic patients undergoing biopsy, and no significant differences in indication for index liver biopsy were identified (Supporting Table 1).

Histological Outcomes. Histology was read under coded assignment in 49 T1DM patients, 45 T2OH patients, and 46 T2IN patients with both available histology slides for analysis, as well as the absence of metastatic malignancy (see Table 3). Steatosis was present in 53.1% of included T1DM patients, significantly less than T2OH patients (53.1% vs. 84.4%; $P = 0.002$), but no different to T2IN patients (53.1% vs. 54.3%, $P = 1.00$). Markers of histological severity were more prevalent in T2OH patients, compared to patients with type 1 diabetes, including hepatocyte ballooning (53.1% vs. 88.9%; $P < 0.001$), any fibrosis

Table 2. Common Primary Clinicopathological Diagnoses After Index Liver Biopsy

Diagnosis	T1DM Cohort (n = 57)	T2OH Cohort (n = 57)	*P* Value (T1DM vs. T2OH)	T2IN Cohort (n = 57)	*P* Value (T1DM vs. T2IN)
NAFLD, n (%)	11 (19.3)	23 (40.4)	**0.02**	16 (28.1)	0.38
Metastatic malignancy, n (%)	7 (12.3)	8 (14.0)	1.00	9 (15.8)	0.78
Alcoholic liver disease, n (%)	6 (10.5)	8 (14.0)	0.77	4 (7.0)	0.74
Autoimmune liver disease, n (%)	7 (12.3)	2 (3.5)	0.16	3 (5.3)	0.32
Hepatitis C, n (%)	7 (12.3)	2 (3.5)	0.16	5 (8.8)	0.76
Hepatic glycogenosis, n (%)	8 (14.0)	1 (1.8)	**0.03**	1 (1.8)	**0.03**

Categorical variables are displayed as n (%) and have been compared using Fisher's exact test. *P* values $\leq$0.05 denote statistically significant differences and are highlighted in bold.

prevalence (73.5% vs. 93.3%; $P = 0.01$), and fibrosis percentage (4% vs. 5.5%; $P = 0.022$). The histological prevalence of NASH, as diagnosed by NASH Clinical Research Network criteria[23] (steatosis, NAS score $\geq$3, and the absence of an alternative explanation for steatosis), was 20.4% in the T1DM cohort—this was significantly lower than T2OH patients (44.4%; $P = 0.02$), but not different to T2IN patients (34.8%; $P = 0.17$). On unadjusted logistic regression analysis (Table 4), type 1 diabetes was associated with a reduced risk of steatosis (odds ratio [OR]: 0.2; 95% confidence interval [CI]: 0.1-0.6; $P = 0.002$), and any fibrosis (OR, 0.2; 95% CI: 0.1-0.7; $P = 0.017$), compared to the T2OH cohort, but not advanced fibrosis (OR, 0.5; 95% CI: 0.2-1.2; $P = 0.112$). After adjusting for age at liver biopsy and obesity, the effect on steatosis was not altered and stayed significant (OR, 0.2; 95% CI: 0.1-0.7; $P = 0.007$), whereas the effect on any fibrosis became insignificant (OR, 0.4; 95% CI 0.1-2.1; $P = 0.306$). There were no significant differences between the T1DM and T2IN cohort for any of these histological features on either uni- or multivariate analysis.

Longitudinal Outcomes. Cirrhosis was diagnosed in 14 patients (24.6%) with type 1 diabetes from index biopsy or during longitudinal follow-up, resulting from autoimmune liver diseases (5 patients), alcoholic liver disease (5 patients), chronic hepatitis C (3 patients), and NASH (1 patient). Cumulative follow-up since diabetes diagnosis were 1,175.96, 679.58, and 864.92 person-years in the T1DM, T2OH, and T2IN cohorts, respectively. Overall survival and incidence of cirrhosis and PH in each cohort since diabetes diagnosis are shown in Figs. 1 and 2. Survival and PH incidence were not statistically different comparing the T1DM cohort with T20H patients (log-rank *P* values: 0.755 and 0.223, respectively) and T2IN patients (*P* values: 0.117 and 0.213, respectively). Cirrhosis incidence was significantly lower in the T1DM cohort, compared to T2IN patients (log-rank *P* value: 0.032) and trended toward being lower than T2OH patients (*P* value: 0.059). HCC occurred during follow-up in 2 patients with type 1 diabetes, 4 patients from the T2OH cohort, and 3 patients from the T2IN cohort.

On unadjusted Cox's proportional hazard regression analysis, type 1 diabetes was associated with a reduced incidence of cirrhosis, compared to the T2IN cohort (hazard ratio [HR]: 0.4; 95% CI: 0.2-1.0; $P = 0.037$) and trended toward reduced cirrhosis incidence, compared to the T2OH cohort (HR, 0.5; 95% CI: 0.2-1.0; $P = 0.065$; Table 5). After adjusting for age of diabetes diagnosis, obesity, hazardous alcohol use, and Hepatitis C, the T1DM cohort was no longer significantly associated with reduced cirrhosis incidence (HR, 0.6; 95% CI: 0.2-1.7; $P = 0.330$; and HR, 0.6; 95% CI: 0.2-2.2; $P = 0.466$; respectively). PH incidence in the T1DM cohort was not significantly different from either type 2 diabetes cohort on either unadjusted or adjusted analysis (Table 6).

Cirrhosis incidence of the entire DIAMOND database for patients with type 1 diabetes, compared to UK general population data, is shown in Table 7. We excluded 79 patients from the database without a recorded diabetes diagnosis date and 6 from the T1DM cohort (3 patients with incident cirrhosis before 25 years of age and 3 with cirrhosis diagnosed within a year of type 1 diabetes diagnosis). Cumulative follow-up since diabetes diagnosis was 75,941 person-years for all patients with type 1 diabetes on the DIAMOND database. The incidence rate ratio (IRR) for cirrhosis in type 1 diabetic patients of all age groups was no different from the general population (IRR, 0.721; 95% CI: 0.357-1.453). However, there was a trend toward significance comparing cirrhosis incidence in patients with type 1 diabetes under 55 years of age with the general population (IRR, 1.875; 95% CI: 0.936-3.757).

Discussion

The prevalence of elevated liver enzymes has been reported to be higher in association with type 1 diabetes than in the general population[19,20]; NAFLD, as

Table 3. Histological Comparison of Diabetes Cohorts at Index Biopsy

	T1DM Cohort	T2OH Cohort	*P* Value (T1DM vs. T2OH)	T2IN Cohort	*P* Value (T1DM vs. T2IN)
No. of patients*	49	45	–	46	–
NAS score (%)					
0-2	22 (44.9)	10 (22.2)	0.13	19 (41.3)	0.82
3-4	14 (28.6)	16 (35.6)		13 (28.3)	
5-8	13 (26.5)	19 (42.2)		14 (21.7)	
Steatosis (%)					
0	23 (46.9)	7 (15.6)	**0.002**	21 (45.7)	1.00
1	10 (20.4)	16 (35.6)	0.14	13 (28.3)	0.51
2	10 (20.4)	17 (37.8)	1.00	8 (17.4)	0.74
3	6 (12.2)	5 (11.1)		4 (8.7)	
0 vs. 1-3	26	38		25	
0-1 vs. 2-3	16	22		12	
0-2 vs. 3	6	5		4	
Lobular inflammation (%)					
0	18 (36.7)	10 (22.2)	0.18	10 (21.7)	0.12
1	19 (38.8)	25 (55.6)	0.81	22 (47.8)	0.65
2	8 (16.3)	7 (15.6)	1.00	8 (17.4)	0.52
3	4 (8.2)	3 (6.7)		6 (13.0)	
0 vs. 1-3	31	35		36	
0-1 vs. 2-3	12	10		14	
0-2 vs. 3	4	3		6	
Ballooning					
0	23 (46.9)	5 (11.1)	**<0.001**	16 (34.8)	0.21
1	12 (24.5)	16 (35.6)	**0.01**	9 (19.6)	0.14
2	13 (26.5)	24 (53.3)		19 (41.3)	
0 vs. 1-2	25	40		30	
0-1 vs. 2	13	24		19	
NASH†	10 (20.4)	20 (44.4)	**0.02**	16 (34.8)	0.17
Fibrosis stage (%)					
0	13 (26.5)	3 (6.7)	**0.01**	7 (15.2)	0.21
1	15 (30.6)	12 (26.6)	**0.02**	8 (17.4)	**0.02**
2	7 (14.3)	10 (22.2)	0.13	9 (19.6)	0.06
3	9 (18.4)	8 (17.8)	0.06	8 (17.4)	**0.02**
4	5 (10.2)	12 (26.7)		14 (30.4)	
0 vs. 1-4	36	42		39	
0-1 vs. 2-4	21	30		31	
0-2 vs. 3-4	14	20		22	
0-3 vs. 4	5	12		14	
% fibrosis	4.0 (2.0-8.0)	5.5 (4.0-14.25)	**0.022**	7.5 (3-15)	
Pericellular fibrosis (%)					
0	29 (59.2)	11 (24.4)	**<0.001**	18 (39.1)	0.07
1	13 (26.5)	21 (46.7)	0.13	17 (37.0)	0.30
2	4 (8.2)	10 (22.2)	1.00	10 (21.7)	0.62
3	3 (6.1)	3 (6.7)		1 (2.2)	
0 vs. 1-3	20	34		28	
0-1 vs. 1-2	7	13		11	
0-2 vs. 3	3	3		1	
Iron (%)					
0	40 (81.6)	37 (82.2)	1.00	37 (80.4)	1.00
1	3 (6.1)	7 (15.6)		5 (10.9)	
2	5 (10.2)	1 (2.2)		2 (4.3)	
3	1 (2.0)	0 (0)		2 (4.3)	
0 vs. 1-3	9	8		9	
Portal inflammation (%)					
0	14 (28.6)	5 (11.1)	**0.04**	4 (8.7)	**0.02**
1	19 (38.8)	21 (46.7)	0.40	17 (37.0)	**0.04**
2	9 (18.4)	14 (31.1)	0.76	15 (32.6)	0.43
3	7 (14.3)	5 (11.1)		10 (21.7)	
0 vs. 1-3	35	40		42	
0-1 vs. 2-3	16	19		25	
0-2 vs. 3	7	5		10	

Table 3. *Continued*

	T1DM Cohort	T2OH Cohort	*P* Value (T1DM vs. T2OH)	T2IN Cohort	*P* Value (T1DM vs. T2IN)
Nuclear glycogenosis (%)					
0	25 (51.0)	14 (31.1)	0.06	23 (50.0)	1.00
1	10 (20.4)	14 (31.1)		13 (28.3)	
2	11 (22.4)	13 (28.9)		5 (10.9)	
3	3 (6.1)	4 (8.9)		5 (10.9)	
0 vs. 1-3	24	31		23	
Cytoplasmic glycogenosis (%)					
0	39 (79.6)	43 (95.6)	**0.03**	45 (97.8)	**0.008**
1	2 (4.1)	1 (2.2)		0 (0)	
2	1 (2.0)	1 (2.2)		0 (0)	
3	7 (14.3)	0 (0)		1 (2.2)	
0 vs. 1-3	10	2		1	

Comparison of categorical variables has been performed using Fisher's exact test. *P* values $\leq$0.05 denote statistically significant differences and are highlighted in bold.

*Patients excluded from analysis if histology performed for nonhepatic malignancy staging (T1DM, n = 7; T2OH, n = 10; T2IN, n = 9) or background liver tissue unavailable for histological analysis (T1DM, n = 1; T2OH, n = 2; T2OH, n = 2).

†Defined as NAS score $\geq$3 in the presence of steatosis and absence of alternative cofactor for CLD.

determined by US of the liver has also been considered very common in these patients.[21,26] In a longitudinal cohort of patients with type 1 diabetes, we have demonstrated a substantial burden of biopsy-proven CLD; 86% of biopsied patients had histological evidence of CLD, including the presence of fibrosis in 74%, and nearly 25% of patients developed cirrhosis during longitudinal follow-up from diabetes diagnosis. In addition, we have found that in those under the age of 55 years, type 1 diabetes tended to be associated with a 1.875-fold increased incidence of liver cirrhosis, when compared to the general population. During follow-up, liver-related adverse outcomes were similar in type 1 diabetes, compared to those with type 2 diabetes, on either treatment modality (oral hypoglycemic agents or insulin), after adjusting for, in particular, the younger age of diabetes diagnosis in the type 1 diabetes cohort.

Previous histological analysis of patients with type 1 diabetes and liver disease have been limited to small case series of patients with hepatic glycogenosis,[17,18,27,28] a benign and reversible condition characterized by acute hepatomegaly, abdominal pain, and gross hyperlipidemia exacerbated by poorly controlled insulin deficiency. In only two instances was coexistent

Table 4. Predictors of Hepatic Steatosis and Fibrosis by Uni- and Multivariate Logistic Regression

Steatosis (Any Grade): Univariate Analysis				Steatosis (Any Grade): Multivariate Analysis*			
Cohort	**OR (95% CI)**	**Reciprocal of OR (95% CI)**	***P* Value**	**Cohort**	**OR (95% CI)**	**Reciprocal of OR (95% CI)**	***P* Value**
T2OH vs. T1DM	4.8 (1.8-12.8)	0.2 (0.1-0.6)	**0.002**	T2OH vs. T1DM	4.7 (1.5-14.4)	0.2 (0.1-0.7)	**0.007**
T2IN vs. T1DM	1.1 (0.5-2.4)	0.9 (0.4-2.1)	0.900	T2IN vs. T1DM	1.0 (0.3-3.0)	1.0 (0.3-2.7)	0.935
Fibrosis (Any Grade): Univariate Analysis				**Fibrosis (Any Grade): Multivariate Analysis***			
Cohort	**OR (95% CI)**	**Reciprocal of OR (95% CI)**	***P* Value**	**Cohort**	**OR (95% CI)**	**Reciprocal of OR (95% CI)**	***P* Value**
T2OH vs. T1DM	5.1 (1.3-19.2)	0.2 (0.1-0.7)	**0.017**	T2OH vs. T1DM	2.2 (0.5-10.6)	0.4 (0.1-2.1)	0.306
T2IN vs. T1DM	2.0 (0.7-5.6)	0.5 (0.2-1.4)	0.181	T2IN vs. T1DM	1.1 (0.3-4.2)	0.9 (0.2-3.3)	0.865
Fibrosis ($\geq$F3 Grade†): Univariate Analysis				**Fibrosis ($\geq$F3 Grade†): Multivariate Analysis***			
Cohort	**OR (95% CI)**	**Reciprocal of OR (95% CI)**	***P* Value**	**Cohort**	**OR (95% CI)**	**Reciprocal of OR (95% CI)**	***P* Value**
T2OH vs. T1DM	2.0 (0.8-4.7)	0.5 (0.2-1.2)	0.112	T2OH vs. T1DM	1.6 (0.6-4.2)	0.6 (0.2-1.8)	0.392
T2IN vs. T1DM	2.3 (1.0-5.4)	0.4 (0.2-1.0)	0.055	T2IN vs. T1DM	1.6 (0.6-4.6)	0.6 (0.2-1.8)	0.389

P values $\leq$0.05 denote statistically significant differences and are highlighted in bold.

*Corresponds to adjustment for patient age at time of index liver biopsy and presence of obesity.

†Refers to bridging fibrosis and cirrhosis.

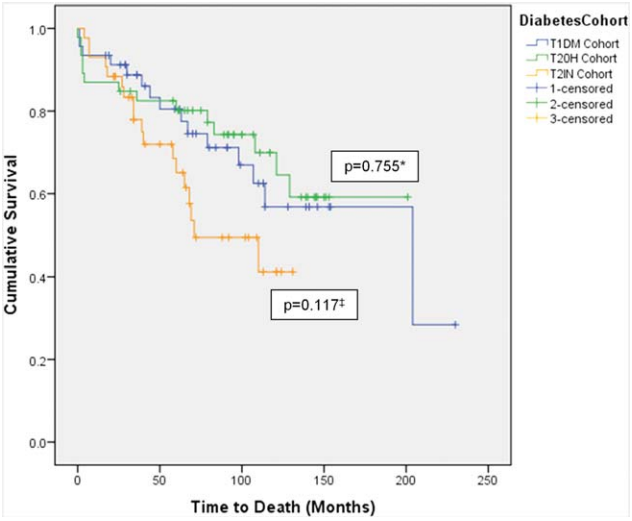

Fig. 1. Kaplan-Meier curve demonstrating survival following index liver biopsy. Individuals stratified by diabetes cohort (T1DM, T2OH, and T2IN, respectively). Outcomes compared by log-rank test with pair-wise comparison between stratified cohorts. *P* values ≤0.05 denote statistically significant differences and are highlighted in bold. **P* value comparing T1DM and T2OH cohorts; ‡*P* value comparing TIDM and T2IN cohorts.

NAFLD or fibrosis histologically reported. Subsequently, Targher et al.'s evaluations of patients with type 1 diabetes demonstrated a 44.4%-54.9% prevalence of US-based diagnosis of NAFLD in their secondary care clinics.[21,29] US has both a relatively poor sensitivity and specificity in diagnosing NAFLD in type 1 diabetes; it has limited ability to detect fatty infiltration when this affects less than one third of hepatocytes,[30] and it is not able to distinguish steatosis from glycogenosis.[18] We have demonstrated that 53.1% of patients with type 1 diabetes who had undergone liver biopsy had histological evidence of steatosis and 20.4% met the histological criteria for NASH—not statistically different from matched patients with type 2 diabetes requiring insulin therapy. The comparative histological findings and longitudinal outcomes, compared to type 2 diabetic patients, and a trend toward a 1.875-fold increased incidence of liver cirrhosis, when compared to the general population, suggests that the presence of type 1 diabetes may be an important cofactor for progressive CLD.

It is important to note that only biopsy-proven patients with cirrhosis were identified by our database search; cirrhosis incidence and prevalence in our type 1 diabetic population may therefore be an underestimate, missing patients diagnosed on clinical grounds alone, or by radiological or other noninvasive biomarker methods. Nonetheless, we have found that type 1 diabetes tended to be associated with a near 1.9-fold increase of cirrhosis incidence, compared to the general UK population, when those under 55 years are considered. However, we excluded 3 T1DM patients who had incident cirrhosis before the age of 25 years to allow direct age-standardized comparison to the GPRD UK population data,[25] which included those 25 years of age or older. Regardless of the statistical significance, the effect size with the sufficient follow-up period in our studied T1DM cohort (a median age of only 43.7 years) emphasizes that cirrhosis incidence in this cohort is clinically important.

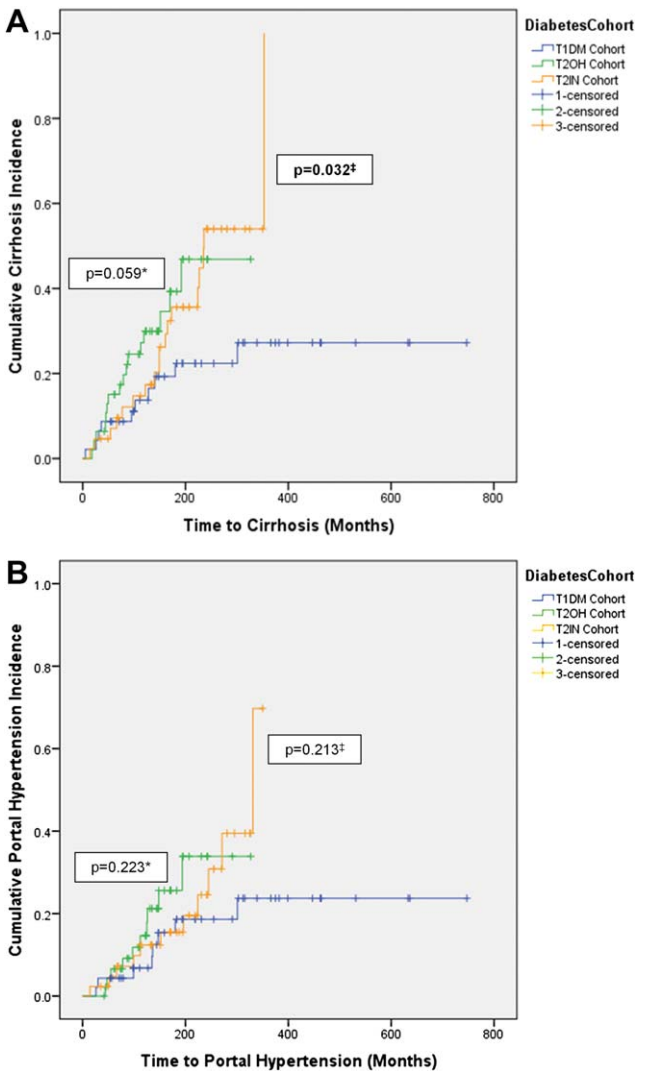

Fig. 2. Kaplan-Meier curve demonstrating cumulative incidence of cirrhosis (A) and PH (B) after diagnosis of diabetes. Individuals stratified by diabetes cohort (T1DM, T2OH, and T2IN, respectively). Outcomes compared by log-rank test with pair-wise comparison between stratified cohorts. *P* values ≤0.05 denote statistically significant differences and are highlighted in bold; **P* value comparing T1DM and T2OH cohorts; ‡*P* value comparing TIDM and T2IN cohorts.

Table 5. Predictors of Developing Cirrhosis by Uni- and Multivariate Proportional Hazard Modeling

Univariate Analysis				Multivariate Analysis*			
Variable	**HR (95% CI)**	**Reciprocal of HR (95% CI)**	***P* Value**	**Variable**	**HR (95% CI)**	**Reciprocal of HR (95% CI)**	***P* Value**
T2OH vs. T1DM	2.2 (1.0-4.9)	0.5 (0.2-1.0)	0.065	T2OH vs. T1DM	1.6 (0.5-5.4)	0.6 (0.2-2.2)	0.466
T2IN vs. T1DM	2.3 (1.0-5.1)	0.4 (0.2-1.0)	**0.037**	T2IN vs. T1DM	1.7 (0.6-4.8)	0.6 (0.2-1.7)	0.330

P values $\leq$0.05 denote statistically significant differences and are highlighted in bold.

*Corresponds to adjustment for patient age at time of diabetes diagnosis, presence of obesity, hazardous alcohol use (defined as >14 units of ethanol per week for women and >21 units of ethanol per week for men), and hepatitis C infection (defined as any previous history of hepatitis C exposure).

Table 6. Predictors of Developing PH by Uni- and Multivariate Proportional Hazard Modeling

Univariate Analysis				Multivariate Analysis*			
Variable	**HR (95% CI)**	**Reciprocal of HR (95% CI)**	***P* Value**	**Variable**	**HR (95% CI)**	**Reciprocal of HR (95% CI)**	***P* Value**
T2OH vs. T1DM	1.8 (0.7-4.7)	0.6 (0.2-1.5)	0.229	T2OH vs. T1DM	1.1 (0.3-4.8)	0.9 (0.2-3.9)	0.891
T2IN vs. T1DM	1.8 (0.7-4.6)	0.6 (0.2-1.4)	0.219	T2IN vs. T1DM	1.2 (0.3-4.3)	0.8 (0.2-3.0)	0.792

P values $\leq$0.05 denote statistically significant differences and are highlighted in bold.

*Corresponds to adjustment for patient age at time of diabetes diagnosis, presence of obesity, hazardous alcohol use (defined as >14 units of ethanol per week for women and >21 units of ethanol per week for men), and hepatitis C infection (defined as any previous history of hepatitis C exposure).

Type 1 diabetes is primarily an insulin-deficient state. Therefore, its association with NAFLD, NASH, and fibrogenesis is counterintuitive; hence, the underlying mechanisms are poorly understood. However, multiple studies utilising the hyperinsulinemic-euglycemic clamp test have demonstrated both whole-body[31] and hepatic IR[32,33] in patients with type 1 diabetes, although it is worth noting that glycemic control of patients included in these initial studies was poor. Interestingly, Bergman et al.[34] confirmed hepatic and skeletal muscle IR in 25 patients with type 1 diabetes with adequate glycemic control (mean *hemoglobin A1c*: 7.7%) and similar metabolic characteristics to healthy controls. This suggests that IR in type 1 diabetes may be one of the mechanisms underlying steatosis and hepatic fibrosis. In the current study, though NAFLD prevalence in biopsied patients with type 1 diabetes was high, only 1 patient with NAFLD developed cirrhosis. Despite insulin therapy, transient, prolonged hyperglycemia in patients with type 1 diabetes is not uncommon and is a feasible cofactor for fibrosis both in the presence and absence of NAFLD. Glucose, in particular, is a major source of acetyl-coenzyme A for TG production. Glucose can also act through carbohydrate response element-binding protein, which regulates both glycolytic and lipogenic enzymes in hepatocytes, hence,playing a central role in coupling these two pathways.[35,36] Additionally, hyperglycemia stimulates the transcription of connective tissue growth factor (CTGF),[37] which appears to be responsible, in part, for development of hepatic fibrosis,[37] and CTGF blockade inhibits hepatic stellate cell activation in rat models of hepatic fibrosis.[38,39] Mechanisms underlying the development of steatosis, hepatic fibrosis both in the presence and absence of steatosis, and their subsequent progression need further evaluation.

The current study has some limitations. First, this was a retrospective analysis, and the findings should be interpreted accordingly. However, both the DIAMOND baseline characteristic and longitudinal patient data, as well as histological data, were collected prospectively as part of standard care. As previously described, longitudinal data were present for 95% of all the patients with type 1 diabetes in the region. Therefore, we are confident that we have correctly identified the vast majority of biopsy-proven cirrhosis in the regional type 1 diabetes population. Second, the diagnosis of type 1 diabetes was based on clinical criteria, made by specialist diabetologists in secondary care, and pancreatic autoimmunity

Table 7. Cirrhosis Incidence Comparing T1DM Cohort and UK General Population Data

	DIAMOND Type 1 Diabetes Data		GPRD General Population Data [27]		
Age, years	**Cases**	**Person-Years***	**Cases**	**Person-Years***	**IRR (95% CI)**
$\geq$25	8	75,941	3,360	23,093,805	0.721 (0.357-1.453)
25-54	8	43,876	1,399	14,391,466	1.875 (0.936-3.757)

Crude IRR were calculated using the formula $((A_1/T_1)/(A_0/T_0))$ A=number of cases, T=total cohort follow-up time (person-years), 1=Type 1 diabetes cohort and 0=GPRD general population data. Confidence intervals (95%) were subsequently calculated using the method described by Rosner. [28]

*Corresponds to cumulative time at risk in years for the chosen study populations.

was not consistently demonstrated in all cases. Whereas we clarified, to our best knowledge, that the individual patients making up the type 1 diabetes cohort had been correctly classified, it is possible that bidirectional misclassification with type 2 diabetes and early insulin requirement may have occurred at the time of initial diabetes diagnosis. It is worth noting that the T1DM and T2IN cohorts in this study had vastly different demographic and metabolic characteristics to each other, as one would expect with differing contributions of insulin deficiency and resistance, and therefore we are confident that any disease misclassification is likely to be small. Last, there is likely to be a selection bias, particularly with type 2 diabetics included in the study; only an estimated 25% of all patients with type 2 diabetes undergo review in secondary care and hence included in the DIAMOND database. Patients with type 2 diabetes requiring secondary care intervention, particularly those in the T2OH cohort, are likely to be those with multiple comorbidities or difficult-to-control hyperglycemia. These patients are likely to have a greater prevalence of both significant liver disease and adverse longitudinal outcomes, compared to the region's entire type 2 diabetic population. Although we did not directly compare the type 2 diabetes cohorts together, this is likely to explain the evident increased severity of histologically proven CLD in T2OH versus T2IN patients, which is in contrast to previous large observational studies.[14]

In conclusion, we found that type 1 diabetes is associated with a substantial burden of CLD and its complications, with a 1.875-fold increased cirrhosis incidence, although this was not statistically significant, compared to the general population, in selected age groups. Further investigations should focus on the risk factors associated with CLD in patients with type 1 diabetes and intervention that detect these early and prevent liver disease progression in these patients.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website.