Prevalence of Non-alcoholic Fatty Liver Disease and Its Associated Factors in Admixed Individuals With Type 1 Diabetes: a Cross-sectional Study in a Tertiary Care Center in Brazil

Bianca Senger Vasconcelos Barros (bibsrj@gmail.com)
Universidade do Estado do Rio de Janeiro - Campus Vila Isabel https://orcid.org/0000-0001-9528-2378
Fernanda Cruz Monteiro
Universidade do Estado do Rio de Janeiro
Carlos Terra
Universidade do Estado do Rio de Janeiro
Marilia Brito Gomes
Universidade do Estado do Rio de Janeiro

Research

Keywords: type 1 diabetes, fatty liver, steatosis, elastography, ultrasound, metabolic syndrome

Posted Date: January 5th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-138525/v1

Version of Record: A version of this preprint was published on March 19th, 2021. See the published version at https://doi.org/10.1186/s13098-021-00649-0.

Abstract

Background: Data on non-alcoholic fatty liver disease (NAFLD) in admixed individuals with type 1 diabetes (T1D) is lacking. We investigated NAFLD in an admixed population with T1D from a tertiary care center in Brazil.

Methods: Ninety-five participants with T1D, aged 39 ± 13 years, with disease duration of 21 ± 9 years, being 55 (57.9%) females, from a university hospital in Rio de Janeiro, were screened for NAFLD with hepatic ultrasound (US) and transient elastography (TE).

Results: Prevalence of NAFLD was, respectively, 12.6% and 16.8% when US and TE were used. Fibrosis was present in 8% of participants. A total of 31.6% of participants had at least one of the hepatic exams altered, which was associated with higher anthropometric measurements, presence of metabolic syndrome and higher triglycerides levels, even within the normal range.

Conclusion: In our study, prevalence of NAFLD in US approximates from the one found with TE. Screening should be reserved for participants with T1D and metabolic syndrome, as this was the main factor associated with NAFLD. Triglycerides levels were the only component of metabolic syndrome associated with NAFLD. Further studies are necessary to determine the best screening strategy for NAFLD in individuals with T1D from admixed populations.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is one the most frequent liver diseases and it is associated with obesity, insulin resistance, type 2 diabetes, enhanced cardiovascular risk, and risk for hospitalization and death due to liver complications such as cirrhosis and hepatocellular carcinoma(1). NAFLD involves a range of alterations including steatosis, steatohepatitis, fibrosis, and cirrhosis(2). Fibrosis is a marker of liver complication and should be assessed for prognosis(3). Global prevalence of NAFLD is around 25% (1,2,4). Although we have a worldwide overweight and obesity epidemic (5) which includes individuals with type 1 diabetes (T1D)(6), NAFLD has not been the focus of many studies with T1D, resulting in a broad range of prevalence (2,7–13). Although portal hyperinsulinemia and insulin resistance have been implicated in the development of NAFLD, the pathogenesis in T1D is controversial. In these individuals, exogenous insulin is administered and it achieves high peripherical concentration but low portal concentration. This may prevent hepatic lipogenesis and development of NAFLD in T1D(7,14). However, alternative pathogenic pathways, such as activation of lipogenesis in hyperglycemic states, may explain how NAFLD could be also a complication in T1D(15).

The aim of this study was to determine NAFLD prevalence by two methods and its associated factors in admixed individuals with T1D from Brazil.

2. Subjects, Materials, And Methods

2.1 Study design

This was a cross-sectional study conducted between 2016 and 2020, with individuals with T1D, treated by an endocrinologist in the Diabetes Unit at Policlínica Piquet Carneiro, a public tertiary health center. They were consecutively invited to participate in the study during regular visits. We included individuals with T1D, aged at least 13 years old, diagnosed by a physician through classical clinical findings (hyperglycemia, polyuria, weight loss, polydipsia, polyphagia and dependency on insulin therapy since diagnosis), that were assisted for at least 6 months in our center. The exclusion criteria were: being pregnant or breastfeeding at the time of inclusion; known liver disease; daily alcohol ingestion above 20 g for women or 30 g for men; acute infectious process, hospitalization, or ketoacidosis in the 3 months prior to recruitment. All participants or their caregivers signed informed consent and study was approved by local ethics committee.

2.2 Data collection

Data were collected on gender, current age, diabetes duration, years of school attendance, self-reported color-race (White, Black, Brown, Asian or Indigenous, as recommended by Brazilian Institute of Geography and Statistics)(16), alcohol consumption, type of insulin and daily dose, use of other medications and comorbidities. Clinical variables included weight (in kilograms), height (in centimeters), body mass index (BMI), blood pressure (BP), waist circumference (WC; determined at half the distance between the last costal arch and the iliac crest), hip circumference (HC), and random capillary glucose. Laboratory measurements were obtained, after an overnight fast: fasting plasma glucose, glycated hemoglobin A1c (HbA1c; measured with high- performance liquid chromatography), urea, creatinine, total cholesterol, high-density lipoprotein cholesterol (HDL), triglycerides, low-density lipoprotein cholesterol (LDL) calculated by Friedewald´s equation, alanine aminotransferase (ALT), aspartate aminotransferase (ALT), ultrasensitive C-reactive protein (CRP), creatine phosphokinase (CPK), gamma-glutamyl transferase (GGT) and uric acid. For ALT and AST, we considered normal values of < 25 U/L for women and < 33 U/L for men (17). Estimated glomerular filtration rate (eGFR) was calculated with CKD-EPI formula. Fatty liver index (FLI) was calculated to determine the risk of fatty liver(18). Participants with FLI ≥ 60 were classified at high risk and participants with values < 30 were at low risk for fatty liver. Values between 30 and 60 were undetermined risk. Viral hepatitis B with HBs antigen and hepatitis C with anti-HCV, were measured by electrochemiluminescence technique.

2.3 Evaluation of liver steatosis and fibrosis

Participants underwent two hepatic image methods: ultrasound (US) and liver transient elastography (TE). US was performed by a radiologist after 6 hours of fasting. Steatosis was detected through observation of diffuse hyperechogenicity of the liver in comparison to kidneys, attenuation of ultrasound beam, and difficulty in visualizing intrahepatic vessels (19). TE was performed with FibroScan® 502 (Echosens, Paris, France) by an experienced hepatologist, after participants fasted for 2 to 4 hours. XL probe was selected for participants with BMI > 30 kg/m^2 and distance skin-liver capsule ≥ 25 mm. M probe was selected for remaining participants. Steatosis stage was defined by categories of controlled

attenuation parameter (CAP): S0: CAP < 248 dB/m; ≥ S1: 248–267 dB/m; ≥S2: 268–279 dB/m; ≥S3: ≥ 280 dB/m (20). Fibrosis status was defined by categories of liver stiffness measurement (LSM): F0-F1: LSM < 7.0 kPa; F2: 7.0-8.7 kPa; F3: 8.8–10.3 kPa; F4 > 10.3 kPa (21). CAP results ≥ S1 were considered steatosis and TE results ≥ F2 were considered with significant fibrosis. All participants had at least 10 valid measurements, a success rate above 60% and interquartile range/median ratio for LSM under 30%. Both imaging investigators had no access to clinical and laboratory data from participants.

2.4 Evaluation of metabolic syndrome

Metabolic syndrome (MS) was defined according to the International Diabetes Federation criteria (22). Considering that all participants have diabetes, central obesity plus an additional factor was necessary for diagnosing MS: central obesity: WC ≥ 90 cm in South American men or ≥ 80 cm in South American women; triglycerides ≥ 150 mg/dL (1.7 mmol/L) or on drug therapy for elevated triglycerides; HDL < 40 mg/dL (1.03 mmol/L) in men or < 50 mg/dL (1.29 mmol/L) in women or on drug therapy for low HDL; elevated BP ≥ 130 × 85 mmHg or receiving antihypertensives.

3. Statistical Analysis

Continuous variables are expressed as means ± standard deviations or median [interquartile range]. Categorical variables are expressed as frequencies and percentages. Student' t-tests or Mann-Whitney U test, Chi-square or Fisher's exact test, were used when indicated.

First, we described baseline characteristics of the study population. Second, we compared demographical, clinical and laboratory parameters of the following groups: altered US vs. normal US; altered TE vs. normal TE; and finally altered hepatic image (US and/or TE) vs. normal hepatic images. Spearman's correlation was performed to evaluate which factors were correlated with CAP and LSM measurements. Multivariable logistic regression was done to determine which factors could be associated with the presence of steatosis (steatosis on US and/or steatosis ≥ S1 on TE) and this was the dependent variable in all models. Independent variables were chosen based on statistical significance on exploratory analysis or biological plausibility. In the first model of logistic regression, age, gender, HbA1c and MS were the independent variables. Second model was done to determine which of the components of MS had stronger association with steatosis. Age, gender, HbA1c and WC, HDL, triglycerides and hypertension were the independent variables. Finally, the third model was similar to second model, but also included components of FLI (WC, triglycerides, BMI and GGT) as independent variables. Model fit was assessed through Hosmer and Lemeshow and Omnibus test. Nagelkerke R^2 was calculated and odds ratio (OR) with 95% confidence interval (CI) are expressed as indicated. Differences were considered significant at two-sided $p < 0.05$. All statistical analysis was performed with Statistical Package for Social Sciences (SPSS) 24.0.

4. Results

Ultimately, we recruited 103 participants. Overall, 6.8% (n = 8) were excluded. One patient had missing blood samples and two were misdiagnosed with T1D. Five participants had a diagnosis of hepatitis (two cases of hepatitis C and three of hepatitis B) and were referred to a hepatologist. A total of 95 patients were included in the final analysis.

4.1. Baseline characteristics and prevalence of steatosis

The mean age was 39 ± 13 years, with disease duration of 21 ± 9 years, and 55 (57.9%) participants were female. Forty-eight (50.2%) participants declared to be non-Caucasian (Black or Brown). MS was present in 42 participants (44.2%) and 45 participants (47.4%) were found overweight or obese. The median for HbA1c was 8.6% [IQR 2.1]. Steatosis was diagnosed by ultrasound in 12 participants (12.6%) and, when TE was used, in 16 participants (16.8%). Eight participants (8.4%) showed significant fibrosis. Data shown in Table 1.

Table 1
Baseline characteristics

N	**95**
Age, years	39 ± 13
Female, n (%)	55 (57.9)
Self-reported color-race, n (%)	
Caucasian	47 (49.5)
Black	16 (16.8)
Brown	32 (33.7)
Years of formal education	12 ± 3
Diabetes duration, years	21 ± 9
Overweight, n (%)	31 (32.6)
Obesity, n (%)	14 (14.7)
Metabolic syndrome, n (%)	42 (44.2)
Steatosis on ultrasound, n (%)	12 (12.6)
Steatosis on TE, n (%)	**16 (16.8)**
≥ S1	**13 (12.6)**
≥ S2	**0 (0)**
≥ S3	**3 (3.2)**
Fibrosis ≥ F2 on TE, n (%)	8 (8.4)
F2	3 (3.1)
F3	3 (3.1)
F4	2 (2.1)
Categories of FLI, n (%)	
High risk	16 (16.8%)
Undetermined risk	16 (16.8%)
Low risk	63 (66.3%)

Data are represented as means ± standard deviation, median [interquartile range] or as numbers (percentages); TE: transient elastography; FLI: fatty liver index; HbA1c: glycated hemoglobin. ≥ S1, ≥ S2, ≥ S3 correspond to stages of steatosis and F2, F3 and F4 correspond to stages of fibrosis, determined by TE.

N	95
HbA1c (%)	8.6 [2.1]
(mmol/mol)	70 [25]

Data are represented as means ± standard deviation, median [interquartile range] or as numbers (percentages); TE: transient elastography; FLI: fatty liver index; HbA1c: glycated hemoglobin. ≥ S1, ≥ S2, ≥ S3 correspond to stages of steatosis and F2, F3 and F4 correspond to stages of fibrosis, determined by TE.

4.2. Demographic, clinical, and laboratory parameters according to hepatic images results

We stratified participants according to results on US (altered vs. normal US) and TE (altered vs. normal TE). The group with altered US presented higher rates of MS and higher FLI. Among variables involved in diagnosis of MS, the group with altered US presented higher triglyceride levels and lower HDL in comparison to group with normal US. There was no difference in anthropometric measurements, HbA1c or use of medications. The group with altered TE had higher BMI, WC, HC, waist-to-hip ratio (WHR), FLI, systolic and diastolic blood pressure, higher rates of MS and hypertension, and higher triglycerides levels, in comparison to normal TE group. No other laboratory data differences were found between the two groups of TE. Data shown in Tables 2 and 3.

Table 2
Participants characteristics according to hepatic ultrasound results

	Altered US	Normal US	p value
Demographical and clinical characteristics			
N (%)	12 (12.6)	83 (87.4)	
Age, years	40 ± 13	37 ± 13	0.459
Female gender, n (%)	9 (75.0)	46 (55.4)	0.199
Non-Caucasian, n (%)	8 (66.7)	40 (48.2)	0.232
Years of school attendance	11 ± 3	12 ± 3	0.383
Diabetes duration, years	19 ± 9	21 ± 10	0.406
BMI, kg/m^2	26.7 ± 3.4	25.2 ± 4.1	0.209
WC, cm	89.7 ± 10.0	85.6 ± 11.8	0.253
HC, cm	100.0 ± 7.3	99.3 ± 7.7	0.759
WHR	0.90 ± 0.08	0.86 ± 0.08	0.135
SBP, mmHg	128 ± 18	127 ± 16	0.767
DBP, mmHg	76 ± 9	77 ± 11	0.668
Insulin dose, U/kg	0.82 ± 0.30	0.76 ± 0.31	0.499
Hypertension, n (%)	5 (41.7)	38 (45.8)	0.789
Anti-hypertensive use, n (%)	4 (33.3)	36 (43.4)	0.510
Metformin use, n (%)	2 (16.7)	9 (11.0)	0.567
Statin use, n (%)	5 (41.7)	40 (48.2)	0.672
Acetylsalicylic acid use, n (%)	2 (16.7)	16 (19.3)	0.829
Currently smoking, n (%)	2 (16.7)	4 (4.8)	0.165
Metabolic syndrome, n (%)	10 (83.3)	33 (39.8)	**0.005**
FLI	38 [43]	13 [35]	**0.028**

Altered US refers to steatosis on hepatic ultrasound. Data are represented as means ± standard deviation, median [interquartile range] or as numbers (percentages). BMI: body mass index; WC: waist circumference, HC: hip circumference; WHR: waist-to-hip ratio; SBP: systolic blood pressure, DBP: diastolic blood pressure; HbA1c: glycated hemoglobin; FPG: fasting plasma glucose; HDL: HDL cholesterol; LDL: LDL cholesterol; eGFR: estimated glomerular filtration rate by CKD-EPI equation; ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma-glutamyl transferase; CPK: creatine phosphokinase; CRP: C reactive protein; FLI: fatty liver index.

	Altered US	Normal US	p value
Laboratorial measurements			
HbA1c (%)	8.6 [3.6]	8.6 [2.3]	0.757
mmol/mol	70 [39]	70 [25]	
FPG,mg/dl	116 [160]	160 [135]	0.728
Total cholesterol, mg/dl	154.5 [53.3]	167.0 [65.0]	0.787
HDL-c, mg/dl	38.1 [21.5]	50.0 [30.0]	**0.034**
LDL-c, mg/dl	83.6 [58.5]	94.8 [41.2]	0.375
Triglycerides, mg/dl	139.0 [190.8]	73.0 [60.8]	**0.028**
eGFR, ml/min/1,73 m^2	107 [43]	99 [31]	0.728
Albumin, mg/dl	3.7 ± 0.6	4.0 ± 0.6	0.075
ALT, U/L	11.5 [15.3]	9.0 [7.0]	0.719
AST, U/L	15.5 [14.3]	13.0 [8.0]	0.507
GGT, mg/dl	18.5 [14.3]	19.0 [16.0]	0.848
CPK, mg/dl	100.5 [125.3]	81.0 [86.0]	0.670
CRP, mg/dl	0.4 [0.6]	0.2 [0.4]	0.334
Uric acid, mg/dl	3.6 [0.9]	3.6 [2.0]	0.848

Altered US refers to steatosis on hepatic ultrasound. Data are represented as means ± standard deviation, median [interquartile range] or as numbers (percentages). BMI: body mass index; WC: waist circumference, HC: hip circumference; WHR: waist-to-hip ratio; SBP: systolic blood pressure, DBP: diastolic blood pressure; HbA1c: glycated hemoglobin; FPG: fasting plasma glucose; HDL: HDL cholesterol; LDL: LDL cholesterol; eGFR: estimated glomerular filtration rate by CKD-EPI equation; ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma-glutamyl transferase; CPK: creatine phosphokinase; CRP: C reactive protein; FLI: fatty liver index.

Table 3
Participants characteristics according to transient elastography results

	Altered TE	Normal TE	p value
Demographical and clinical characteristics			
N (%)	22 (23.2)	73 (76.8)	
Age, years	40 ± 11	39 ± 14	0.625
Female gender, n (%)	11 (50.0)	44 (60.3)	0.392
Non-Caucasian, n (%)	9 (40.9)	39 (53.4)	0.303
Years of school attendance	12 ± 4	12 ± 3	0.833
Diabetes duration, years	22 ± 10	20 ± 9	0.448
BMI, kg/m^2	28.9 ± 3.7	24.3 ± 3.6	**< 0.001**
WC, cm	94.9 ± 11.3	83.2 ± 10.4	**< 0.001**
HC, cm	104.5 ± 7.4	97.8 ± 7.0	**< 0.001**
WHR	0.91 ± 0.08	0.85 ± 0.07	**0.003**
SBP, mmHg	135 ± 16	125 ± 16	**0.011**
DBP, mmHg	81 ± 11	76 ± 10	**0.027**
Insulin dose, U/kg	0.75 ± 0.26	0.77 ± 0.33	0.768
Hypertension, n (%)	14 (63.6)	29 (39.7)	**0.048**
Anti-hypertensive use, n (%)	13 (59.1)	26 (35.6)	0.050
Metformin use, n (%)	5 (22.7)	6 (8.2)	0.120
Statin use, n (%)	14 (63.6)	30 (41.1)	0.063
Acetylsalicylic acid use, n (%)	6 (27.3)	12 (16.4)	0.351
Currently smoking, n (%)	1 (4.5)	5 (5.3)	1.000
Metabolic syndrome, n (%)	15 (68.2)	27 (37.0)	**0.010**
FLI	60 [58]	13 [22]	**< 0.001**

Altered TE refers to steatosis and/or fibrosis on transient elastography (TE). Data are represented as means ± standard deviation, median [interquartile range] or as numbers (percentages). BMI: body mass index; WC: waist circumference, HC: hip circumference; WHR: waist-to-hip ratio; SBP: systolic blood pressure, DBP: diastolic blood pressure; HbA1c: glycated hemoglobin; FPG: fasting plasma glucose; HDL: HDL cholesterol; LDL: LDL cholesterol; eGFR: estimated glomerular filtration rate by CKD-EPI equation; ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma-glutamyl transferase; CPK: creatine phosphokinase; CRP: C reactive protein; FLI: fatty liver index.

	Altered TE	Normal TE	p value
Laboratorial measurements			
HbA1c (%)	8.9 [2.8]	8.5 [2.3]	0.717
mmol/mol	74 [30]	70 [25]	
FPG,mg/dl	130 [200]	116 [131]	0.517
Total cholesterol, mg/dl	170.0 [77.3]	161.0 [65.0]	0.880
HDL-c, mg/dl	50.8 [24.5]	47.6 [32.8]	0.880
LDL-c, mg/dl	99.9 [46.1]	91.4 [40.2]	0.383
Triglycerides, mg/dl	89.0 [99.0]	75.0 [62.5]	**0.040**
eGFR, ml/min/1,73 m^2	99 [31]	100 [30]	0.771
Albumin, mg/dl	4.0 ± 0.7	4.0 ± 0.6	0.992
ALT, U/L	10.0 [8.5]	8.0 [7.0]	0.260
AST, U/L	14.5 [8.3]	12.0 [7.5]	0.082
GGT, mg/dl	20.5 [41.3]	19.0 [16.5]	0.596
CPK, mg/dl	119.0 [172.0]	80.0 [86.0]	0.667
CRP, mg/dl	0.3 [0.9]	0.2 [0.4]	0.771
Uric acid, mg/dl	3.8 [1.8]	3.5 [1.7]	0.383

Altered TE refers to steatosis and/or fibrosis on transient elastography (TE). Data are represented as means ± standard deviation, median [interquartile range] or as numbers (percentages). BMI: body mass index; WC: waist circumference, HC: hip circumference; WHR: waist-to-hip ratio; SBP: systolic blood pressure, DBP: diastolic blood pressure; HbA1c: glycated hemoglobin; FPG: fasting plasma glucose; HDL: HDL cholesterol; LDL: LDL cholesterol; eGFR: estimated glomerular filtration rate by CKD-EPI equation; ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma-glutamyl transferase; CPK: creatine phosphokinase; CRP: C reactive protein; FLI: fatty liver index.

When we considered both exams together, the group with altered hepatic image (US and/or TE) had higher BMI, WC, HC, WHR, FLI, CAP measurements and triglycerides, in comparison to the group with normal images. The rate of MS was higher in the group with altered images compared to the group with normal images. There was no difference in other measurements, including HbA1c, transaminases, insulin dose or other medications. Data shown in Table 4.

Table 4
Participants characteristics according to hepatic image results

	Altered hepatic image	Normal hepatic image	p value
N	30	65	
Clinical variables			
Age, years	39 ± 12	39 ± 13	0.995
Female, n (%)	18 (60.0)	37 (56.9)	0.778
Non-Caucasian, n (%)	15 (50.0)	33 (50.8)	0.994
Years of formal education	12 ± 4	12 ± 3	0.498
Diabetes duration, years	21 ± 10	20 ± 9	0.738
BMI, kg/m^2	27.9 ± 3.9	24.2 ± 3.6	**< 0.001**
Waist circumference, cm	92.1 ± 11.2	83.1 ± 10.8	**< 0.001**
Hip circumference, cm	102.4 ± 7.9	97.9 ± 7.1	**0.006**
Waist-to-hip ratio	0.90 ± 0.08	0.85 ± 0.08	**0.003**
Systolic blood pressure, mmHg	131 ± 16	125 ± 16	0.092
Diastolic blood pressure, mmHg	79 ± 11	76 ± 10	0.297
Metabolic syndrome, n (%)	21 (67.7)	21 (30.0)	**0.001**
Insulin dose, U/kg	0.78 ± 0.28	0.76 ± 0.32	0.741
Anti-hypertensive use, n (%)	15 (50.0)	24 (36.9)	0.228
Metformin use, n (%)	5 (16.7)	6 (9.2)	0.292
Statin use, n (%)	16 (53.3)	28 (43.1)	0.351
Acetylsalicylic acid use, n (%)	7 (23.3)	11 (16.9)	0.459
Currently smoking, n (%)	3 (10.0)	3 (4.6)	0.316
Laboratory measurements			
HbA1c, %	8.9 [3.0]	8.5 [2.2]	0.717
mmol/mol	74 [33]	69 [22]	

Altered image refers to steatosis on ultrasound and/or steatosis and/or fibrosis on transient elastography (TE). Data are represented as means ± standard deviation, median [interquartile range] or as numbers (percentages). BMI: body mass index; HbA1c: glycated hemoglobin; eGFR: estimated glomerular filtration rate; ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma-glutamyl transferase; CPK: creatine phosphokinase; CAP: controlled attenuation parameter; LSM: liver stiffness measurement.

	Altered hepatic image	Normal hepatic image	p value
Fasting plasma glucose, mg/dl	130 [175]	116 [137]	0.880
Total cholesterol, mg/dl	168.5 [62.8]	161.0 [66.0]	0.771
HDL cholesterol, mg/dl	45.8 [26.0]	48.6 [32.0]	0.880
LDL cholesterol, mg/dl	95.8 [51.7]	92.6 [39.6]	0.383
Triglycerides, mg/dl	103.0 [103.8]	72.0 [61.5]	**0.040**
eGFR, ml/min/1,73 m^2	103 [33]	99 [29]	0.771
Albumin, mg/dl	3.9 ± 0.7	4.0 ± 0.6	0.268
ALT, U/L	10.0 [11.3]	8.0 [6.5]	0.260
AST, U/L	14.5 [11.5]	12.0 [6.0]	0.082
GGT, U/L	19.5 [17.3]	19.0 [17.5]	0.830
CPK, U/L	100.5 [142.3]	81.0 [85.5]	0.667
C-reactive protein, mg/dl	0.3 [0.7]	0.2 [0.4]	0.771
Uric acid, mg/dl	3.8 [1.5]	3.5 [2.0]	0.383
Fatty liver index	46 [52]	11 [23]	**< 0.001**
TE measurements			
CAP, dB/m	234 ± 51	174 ± 33	**< 0.001**
LSM, kPa	5.6 [3.9]	4.8 [1.8]	0.276

Altered image refers to steatosis on ultrasound and/or steatosis and/or fibrosis on transient elastography (TE). Data are represented as means ± standard deviation, median [interquartile range] or as numbers (percentages). BMI: body mass index; HbA1c: glycated hemoglobin; eGFR: estimated glomerular filtration rate; ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma-glutamyl transferase; CPK: creatine phosphokinase; CAP: controlled attenuation parameter; LSM: liver stiffness measurement.

Also, a sub-analysis showed that participants with altered hepatic image without MS (n = 9) had higher levels of HbA1c (9.5% [IQR 1.5] vs. 8.5% [IQR 2.8]; $p = 0.028$) and higher BMI (25.4 ± 3.47 kg/m^2 vs. 22.6 ± 2.6 kg/m^2; $p = 0.01$) in comparison to participants without altered images and without MS (n = 41). However, in multivariable analysis, only BMI was associated (OR: 1.42, 95% CI 1.07–1.89; *p = 0.016)* with altered image in the group without MS.

In Spearman's correlation we found that CAP was directly correlated with BMI (ρ 0.369; $p < 0.001$), WC (ρ 0.370; $p < 0.001$), HC (ρ 0.343; $p = 0.001$), WHR (ρ 0.248; $p = 0.016$) and FLI (ρ 0.361, $p < 0.001$). No

laboratory parameters were correlated with CAP. Also, LSM was directly correlated with BMI (ρ 0.262; p = 0.010), WC (ρ 0.229; p = 0.026) and WHR (ρ 0.204; *p = 0.047)* and inversely correlated with HDL (ρ -0.360; p < 0.001).

4.3. Descriptive data of the group with altered hepatic image

We explored the characteristics of the 30 participants who had either altered ultrasound and/or altered TE. Refer to Supplementary Table 1 for detailed information.

Of the 30 participants, 21 (70.0%) had MS. Other than diabetes and WC, we found that the most frequent component of MS was hypertension (n = 14/21), followed by low HDL (n = 13/21) and high triglycerides (n = 8/21).

Twelve (40.0%) participants had steatosis on ultrasound and sixteen (53.3%) had steatosis on TE. Five (16.7%) participants had both exams altered.

One patient (3.3%) in the altered hepatic image group had elevated transaminases and this was associated with mild steatosis on ultrasound but with normal TE.

Considering FLI results, eleven (36.7%) participants had high risk, thirteen (43.3%) had low risk and six (20.0%) had undetermined risk in the group with altered image (n = 30). Out of those eleven with high risk, eight (72.7%) had altered TE and three (27.3%) had both images altered. Out of those thirteen with low risk, four (30.8%) had altered US only, eight (61.5%) had altered TE and only and one (7.7%) had both US and TE altered. Out of those six with undetermined risk, one (16.7%) had both US and TE altered, three (50.0%) had altered US only, and two (33.3%) had with altered TE only.

Eight participants had significant fibrosis (≥ F2) on TE with normal liver function tests and were referred to further investigation in the hepatology unit. One (12.5%) of them had mild steatosis on US; the others had normal US and normal CAP on TE. Also, six (75%) of those participants with fibrosis had MS. We performed a sub-analysis comparing the group with fibrosis vs. no fibrosis. Participants with fibrosis had higher WC (95.3 ± 12.7 cm vs. 85.1 ± 11.2 cm; p = 0.017), HC (104.8 ± 9.7 cm vs. 98.8 ± 7.3; p = 0.034) and BMI (29.6 ± 4.9 kg/m^2 vs. 24.0 ± 3.8 kg/m^2; p = 0.002). There was no difference between groups of fibrosis regarding other clinical and laboratory measurements.

4.4. Multivariable logistic regression evaluating associated factors for steatosis by either imaging method

The first model of logistic regression confirmed the association between MS and steatosis on either hepatic image. Nagelkerke R^2 was 16.7% and X^2 was 11.22. Gender, age and HbA1c were not associated to steatosis. In the second model, triglycerides levels were the component of MS associated with risk of steatosis. Second model had a Nagelkerke R^2 of 28% and X^2 was 19.70. In this last model, triglycerides

remained as the only risk factor for steatosis, Nagelkerke R^2 was 32.1% and X^2 was 22.97. Results are shown in Table 5.

Table 5
Multivariable logistic regression for evaluating associated factors for steatosis on hepatic image by either method (ultrasound and/or transient elastography)

Variable	B	Odds ratio	95% Confidence Interval	p value
Model 1				
Age, years	-0.02	0.976	0.935–1.019	0.270
Female	0.04	1.039	0.364–2.963	0.943
HbA1c (%)	-0.02	0.980	0.893–1.075	0.671
Metabolic syndrome	1.71	5.528	1.842–16.592	**0.002**
Model 2				
Age (years)	-0.03	0.974	0.922–1.028	0.338
Female	-0.50	0.606	0.195–1.880	0.386
HbA1c (%)	-0.03	0.975	0.819–1.160	0.773
WC (centimeters)	0.04	1.045	0.991–1.101	0.102
HDL (mg/dl)	-0.01	0.818	0.969–1.025	0.818
Triglycerides (mg/dl)	0.01	1.013	1.002–1.023	**0.015**
Hypertension	0.55	1.724	0.441–6.748	0.434
Model 3				
Age (years)	-0.02	0.985	0.931–1.043	0.614
Female	-0.43	0.653	0.197–2.161	0.485
HbA1c (%)	-0.02	0.983	0.825–1.171	0.850
WC (centimeters)	-0.02	1.007	0.919–1.103	0.883
HDL (mg/dl)	-0.01	0.991	0.961–1.022	0.559
Triglycerides (mg/dl)	0.01	1.014	1.003–1.024	**0.012**
Hypertension	0.22	1.246	0.285–5.451	0.770
BMI (kg/m^2)	0.19	1.215	0.942–1.566	0.134

Steatosis on hepatic image was the dependent variable in all models. Independent variables were chosen based on statistical significance on exploratory analysis or biological plausibility. Model 1 - Adjusted for age, gender, glycated hemoglobin (HbA1c) and metabolic syndrome. Model 2 - Adjusted for age, gender, HbA1c, waist circumference (WC), HDL-cholesterol (HDL), triglycerides and hypertension. Model 3 - Adjusted for age, gender, HbA1c, components of metabolic syndrome (WC, HDL-c, triglycerides and hypertension) and components of fatty liver index [WC, triglycerides, body mass index (BMI) and gamma-glutamyl transferase (GGT)].

Variable	B	Odds ratio	95% Confidence Interval	p value
GGT (mg/dl)	0.01	1.008	0.993–1.023	0.785

Steatosis on hepatic image was the dependent variable in all models. Independent variables were chosen based on statistical significance on exploratory analysis or biological plausibility. Model 1 - Adjusted for age, gender, glycated hemoglobin (HbA1c) and metabolic syndrome. Model 2 - Adjusted for age, gender, HbA1c, waist circumference (WC), HDL-cholesterol (HDL), triglycerides and hypertension. Model 3 - Adjusted for age, gender, HbA1c, components of metabolic syndrome (WC, HDL-c, triglycerides and hypertension) and components of fatty liver index [WC, triglycerides, body mass index (BMI) and gamma-glutamyl transferase (GGT)].

5. Discussion

In our study, prevalence of 12.6% of steatosis was found with US results, and 16.8% of steatosis when we considered only TE results. When we combined hepatic images (US and/or TE), altered results had association with higher rates of MS, FLI and anthropometric measurements. The components of MS associated with steatosis were waist circumference and triglycerides. US was associated mainly with laboratory components of MS, while TE was associated mainly with anthropometric measurements.

The pathogenesis of NAFLD in T1D is controversial. Physiologically, pancreatic insulin is partly cleared in first-pass metabolism on liver, resulting in higher portal insulin levels and lower levels in peripheral circulation(15). Portal hyperinsulinemia is associated with insulin resistance and stimulates lipogenesis and steatosis(3). In T1D, because insulin is administered exogenously, this gradient is lost, which could protect against NAFLD(15). However, alternative pathways have been proposed to explain NAFLD in T1D. ChREBP (Carbohydrate sensitive response element-binding protein) and SREBP-1c (Sterol regulatory element-binding protein 1) are transcription factors that can be activated in the presence of hyperglycemia, independently of hepatic insulin levels, leading to expression of lipogenic genes and promoting fatty liver(3,15). Also, lipoprotein disturbances (such as glycation of apolipoproteins and increased LDL oxidation) may be present in T1D and could result in reduced hepatic exportation of VLDL, leading to NAFLD(15). These metabolic abnormalities can be present even in individuals with T1D and good glycemic control(23). Few studies have investigated the prevalence of NAFLD in T1D, which ranges from 8 to 50%, depending on characteristics of the studied population such as age, frequency of obesity, ethnicity, and method for diagnosis of steatosis (8,10,14,24–26). To our knowledge, this is the first study to access prevalence of NAFLD in a sample of T1D in Brazil, a highly admixed population, with different lifestyle, eating habits and different ethnicity.

NAFLD has a strong association with MS and all of its components(1). Not surprisingly, in our study MS was two times more frequent in the group with altered hepatic image. The most frequent component of MS was hypertension. However, after multivariable adjustment, we found that triglycerides had stronger association with steatosis. Also, when we considered components of FLI, we found risk association with triglycerides levels, even within the normal range. No other factor was associated with steatosis, including glycemic control, transaminases or GGT.

Although FLI was initially developed in comparison to abdominal ultrasound, it has been compared to TE. One study reported that CAP performed better than FLI in detecting steatosis ≥ S2 on liver biopsy(27). This study proposes a CAP cut-off of 310 dB/m to detect steatosis ≥ S2 but it analyzed a population different from ours: only 59% of participants had diabetes and mean BMI was 30 kg/m^2. Therefore, this cut-off may not be applicable to our population. TE is widely used for prognosis assessment with fibrosis stage, but it is still lacking validation for steatosis' diagnosis through CAP. We used cut-off values proposed by de Lédinghen et. al(28) but there is still much discussion regarding optimal cut-off points for CAP(20). Although ultrasound is the preferred initial image for detecting steatosis and TE is usually recommended for fibrosis assessment after steatosis was detected, we chose to perform both methods to see how they would relate to each other(1,29). Although frequency of steatosis found with TE approximates to the frequency found with US, the two imaging methods identified different participants. However, so far, cut-off values of CAP have not been proposed for T1D in comparison to liver biopsy, emphasizing the controversial aspects of this subject in T1D and the need for further studies.

A relevant proportion (31.6%) of our sample had alteration in at least one of the hepatic images and this warrants attention. Some points of our study probably need further investigation, yet to be stablished. Some participants had normal US and altered TE. However, it might be useful to follow prospectively these participants with altered TE, MS and high FLI, analyzing CAP and LSM as continuous variables. Also, we should reinforce metabolic control and weight loss, a real challenge in routine clinical practice.

Our study has some limitations. As previously mentioned, we used two non-invasive methods to detect NAFLD. US is the main tool for screening NAFLD, easily accessible, with low cost, but operator-dependent and with limited sensitivity(19,30). TE, the other method, lacks validation for diagnosis of steatosis. Although we did not have histological confirmation of our findings, the gold-standard exam would be liver biopsy, which is invasive, susceptible to sampling error (30) and inappropriate for screening proposes of our study. Another limitation was the cross-sectional design of the study. Follow-up is necessary to determine how participants with altered hepatic image will evolve.

As strengths of our study we have participants with T1D from an admixed population, which were screened by two methods. The majority of studies with NAFLD in T1D performed only ultrasound(8,10,24–26). Some performed MRI and found lower rates of NAFLD, but this resource is not widely available(7,11,13,14). Also, we did not had access to studies that used TE for steatosis status in T1D so we present novel data. We found two studies that used TE for fibrosis assessment in children and adolescents with T1D, but CAP is not mentioned (31,32).

In conclusion, screening of NAFLD should be considered for T1D with MS and increasing levels of triglycerides, even within the normal range. Diagnosis of NAFLD should be accompanied of measurements to improve metabolic parameters, because of higher cardiovascular risk in this condition. Further prospective studies are necessary to determine the best screening strategy and outcomes in T1D from admixed populations.

Declarations

Funding:

FAPERJ (Fundação do Amparo à Pesquisa do Estado do Rio de Janeiro) [E 26/110.170/2013] and CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico do Brasil) [563753/2010-2].

Competing interests:

On behalf of all authors, the corresponding author states that there is no conflict of interest. The funding sponsor had no role in the design of the study, in the collection, analysis, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

Ethics approval:

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of State University of Rio de Janeiro (03/07/16/No.1.440.347 and 06/16/19/No.3.417.179).

Consent to participate:

All participants signed informed consent.

Availability of data and material:

Data is available upon request to corresponding author.

Author's contributions:

MBG designed the study; BSVB collected the data; FCM and CT performed hepatic images; MBG and BSVB analyzed the data; BSVB, FCM, CT and MBG wrote and reviewed the manuscript.

Acknowledgments:

We thank the funding sponsors, Conselho Nacional de Tecnologia e Desenvolvimento e Fundação do Amparo à Pesquisa do Estado do Rio de Janeiro. We also thank Mrs. Eliete Leão Silva Clemente, for nursing assistance and Mrs. Maria de Fatima Bevilacqua, for laboratory measurements.

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