Screening for hepatic fibrosis and steatosis in Turkish patients with type 2 diabetes mellitus: A transient elastography study

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ABSTRACT

Background/Aims: *Non-alcoholic fatty liver disease is highly prevalent in patients with type 2 diabetes mellitus (T2DM). The aim of the present study was to investigate the potential usefulness of transient elastography (TE), which is a technique that allows measuring both fibrosis and liver fat content simultaneously, as a screening tool for hepatic involvement in Turkish patients with T2DM.*

Materials and Methods: *We obtained liver stiffness measurements (LSMs, as a measure of fibrosis) and controlled attenuation parameter (CAP, as a marker of steatosis) in 124 (46 males and 78 females; mean body mass index (BMI): 33.2±6.6 kg/m^2) Turkish patients with T2DM. The prevalence rates of overweight, obesity, and metabolic syndrome in our sample were 28.2%, 64.5%, and 77.4%, respectively. Probe-specific LSM cut-off values were used to define advanced fibrosis (≥F3) and cirrhosis (F4) (M probe: F3=9.6-11.4 kPa, F4 ≥11.5 kPa and XL probe: F3=9.3-10.9 kPa, F4 ≥11.0 kPa). Mild, moderate, and severe steatosis were defined as CAP 222-232 dB/m, CAP 233-289 dB/m, and CAP ≥290 dB/m, respectively.*

Results: *Advanced fibrosis and cirrhosis were identified in 21 (16.9%) and 10 (8.0%) patients, respectively. TE-defined hepatic steatosis (CAP>222 dB/m) was detected in 117 (94.3%) patients. Mild, moderate, and severe steatosis were identified in 0, 29, and 88 patients, respectively.*

Conclusion: *TE is a useful non-invasive imaging modality to screen for liver involvement in Turkish patients with T2DM. High rates of TE-defined fibrosis and steatosis in our sample reflect the presence of an elevated mean BMI.*

Keywords: *Type 2 diabetes mellitus, non-alcoholic fatty liver disease, fibroscan, prevalence, obesity, metabolic syndrome*

INTRODUCTION

Patients with type 2 diabetes mellitus (T2DM) are at risk for non-alcoholic fatty liver disease (NAFLD)-most likely due to the widespread occurrence of obesity and insulin resistance in this patient group (1). Although NAFLD can lead to advanced fibrosis and cirrhosis (ultimately increasing liver-related mortality) (2), most patients are asymptomatic and typically identified when the liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are found to be increased during routine laboratory examinations (3,4). However, ALT is not invariably elevated in NAFLD, and its levels do not accurately reflect the extent of hepatic fibrosis and fat accumulation (5,6). Routine liver ultrasound can also provide insight into the extent of hepatic fat deposition in T2DM but also does not have sufficient accuracy for identifying, or ruling out, fibrosis (7). Owing to the known limitations of liver biopsy, including its invasive nature and sampling errors, the role of novel non-invasive screening modalities for hepatic fibrosis and steatosis has been a subject of growing interest (8).

Transient elastography (TE) is increasingly being used as a non-invasive, operator-independent, simple-to-perform imaging modality to assess liver stiffness and hepatic fat deposition (9). In this regard, liver stiffness measurement (LSM) and controlled attenuation parameter (CAP) obtained from TE have been shown to be reliable imaging markers of liver fibrosis and steatosis, respectively (10). Recently, studies from Romania (11), Hong Kong (12), Ko-

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rea (13), and France (14) have reported that TE-derived parameters may be a clinically useful screening tool for hepatic fibrosis and steatosis in T2DM. In the present study, since ethnicity has been shown to significantly affect the risk of NAFLD (15,16), we investigate the prevalence of TE-defined hepatic fibrosis and steatosis in a sample of Turkish patients with T2DM.

MATERIALS AND METHODS

Patients

The study was conducted at Marmara University School of Medicine Hospital between August 2015 and August 2017. Patients aged between 18 and 65 years old were included in the study if (1) they had a diagnosis of T2DM according to the American Diabetes Association criteria (17) and (2) they expressed their willingness to undergo TE for LSM and CAP quantification. Exclusion criteria were as follows: (1) chronic liver diseases (viral hepatitis, autoimmune hepatitis, hemochromatosis, primary biliary cirrhosis, Wilson's disease, sclerosing cholangitis, biliary obstruction, alpha-1 antitrypsin deficiency), (2) malignancies, (3) heart failure (New York Heart Association class III-IV), (4) pregnancy, (5) implanted medical devices (e.g., cardiac pacemakers), (6) end-stage renal disease (glomerular filtration rate<15 mL/min/1.73 m^2), (7) alcohol intake >20 g/day in women and >30 g/day in men, (8) use of steatogenic drugs (e.g., estrogens, amiodarone, steroids, and tamoxifen), and (9) measurement failure or unreliable measurements on TE (see below). Clinical and laboratory data were obtained from medical records. Body mass index (BMI) was calculated as weight in kilograms (kg) divided by the square of height in meters (m^2). Overweight and obesity were defined by BMI >25 kg/m^2 and BMI >30 kg/m^2, respectively. Metabolic syndrome was diagnosed according to the Adult Treatment Panel III criteria (18). After 15 min of seated rest, two blood pressure measurements were obtained (1 min apart) from the right arm of the seated patient using an automated sphygmomanometer. The average of the two measurements was used for analysis. Smoking history was collected by a questionnaire. The AST and ALT levels were considered increased if >37 U/L and >40 U/L, respectively. Microalbuminuria and macroalbuminuria were defined as 30-300 mg/g creatinine and >300 mg/g creatinine, respectively (19). Written informed consent was obtained from all patients. The Institutional Review Board of the Marmara University School of Medicine approved the study.

Transient elastography

Patients were required to fast for at least 3 h before imaging. All TE examinations were performed by a single operator as previously described (20,21). LSM values were quantified by a Fibroscan 502 touch (Echosens SA, Paris, France) using the original M or XL probe (according to the patient's body weight or subcutaneous adipose tissue thickness) according to the manufacturer's instructions. After placing the patient in the dorsal decubitus position, the tip of the transducer probe was positioned on the skin between the ribs over the right lobe of the liver. The examination was conducted through the intercostal space on the right lobe of the liver. After identifying the appropriate area of measurement, the acquisition was started with a measurement depth of 25-65 mm for the M probe and 35-75 mm for the XL probe. TE examinations were considered reliable when at least 10 successful acquisitions were obtained, and the interquartile range-to-median ratio of the 10 acquisitions was ≤0.3 (19).

The following probe-specific LSM cut-off values were used to define advanced fibrosis (≥F3) and cirrhosis (F4): M probe: F3=9.6-11.4 kPa, F4 ≥11.5 kPa and XL probe: F3=9.3-10.9 kPa, F4 ≥11.0 kPa) (12). In accordance with the previously reported methodology (19), the CAP value (100-400 dB/m) was the median value of individual measurements. Mild, moderate, and severe steatosis were defined as CAP 222-232 dB/m, CAP 233-289 dB/m, and CAP ≥290 dB/m, respectively (12).

Statistical analysis

Normally distributed continuous data are expressed as mean±standard deviation, whereas median and interquartile range were used for skewed variables. Categorical data are expressed as counts. The association between the continuous variables was tested using the Spearman's correlation coefficient. Multivariable stepwise linear regression analyses were used to identify the independent predictors of CAP and LSM in patients with T2DM. All of the variables were entered into the multivariable model as potential predictors/covariates (Table 1). Comparisons of ALT levels across different TE-defined groups were performed using the Mann-Whitney U test. All statistical analyses were performed using the SPSS 20.0 software (IBM Corp.; Armonk, NY, USA). A p value <0.05 (two-tailed) was considered statistically significant.

RESULTS

Of a total of 127 patients with T2DM who underwent TE examination, 3 (2.3%) were excluded due to unreliable results. Overall, the final study cohort consisted of 124 (46 male and 78 female) patients. The mean BMI of the patients was 33.2±6.6 kg/m^2. Table 1 shows the general characteristics of the study subjects.

Table 1. General characteristics of the 124 patients with type 2 diabetes mellitus

Characteristics	
Sex, male/female	46/78
Age, years	53±7
Body mass index, kg/m2	33.2±6.6
Lean/overweight/obese	9/35/80
Waist circumference, cm	111±13
Hip circumference, cm	113±11
Homeostasis model assessment-insulin resistance	4.6 (2.9-6.5)
Metabolic syndrome, yes/no	96/28
History of hypertension, yes/no	75/49
Systolic blood pressure, mm Hg	139±19
Diastolic blood pressure, mm Hg	78±10
Smoking history, never/former/current	72/52/0
AST, U/L	23 (18-32)
ALT, U/L	26 (20-48)
Increased AST level, yes/no	24/100
Increased ALT levels, yes/no	37/87
Total cholesterol, mg/dL	213±46
HDL cholesterol, mg/dL	49±13
LDL cholesterol, mg/dL	129±40
Triglycerides, mg/dL	146 (109-202)
Microalbuminuria/macroalbuminuria	66/34

Table 2. ALT levels in patients with type 2 diabetes mellitus in relation to transient elastography-defined hepatic fibrosis and steatosis

		Severity of fibrosis	
	No F3/4	≥F3	F4
ALT, U/L	21 (17-29)	39 (23-50)	24 (19-35)
		Severity of steatosis	
	None	Moderate	Severe
ALT, U/L	19 (15-30)	31 (22-47)	34 (20-54)

Hepatic fibrosis

The M and XL probes were used in 115 (92.7%) and 9 (7.3%) patients, respectively. The mean LSM was 7.1±3.4 kPa, with minimum and maximum values of 2.9 and 25.1 kPa, respectively. When probe-specific LSM cut-off values were used, we identified 21 (16.9%) and 10 (8.0%) patients with advanced fibrosis (≥F3) and cirrhosis (F4), respectively. LSM values were found to be significantly correlated with BMI (r=0.41, p<0.001), waist circumference (r=0.36, p<0.001), hip circumference (r=0.32, p<0.001), systolic blood pressure (r=0.23, p=0.01), diastolic blood pressure (r=0.21, p=0.02), and AST (r=0.29, p<0.001). In multivariable stepwise linear regression analyses, BMI was retained as the only independent predictor of LSM (beta=0.35, t=4.1, p<0.001).

Hepatic steatosis

The mean CAP value was 317±54 dB/m, with minimum and maximum values of 184 and 400 dB/m, respectively. TE-defined hepatic steatosis (CAP>222 dB/m) was detected in the large majority of the sample (n=117; 94.3%). Mild, moderate, and severe steatosis were identified in 0, 29, and 88 patients, respectively. Univariate correlation analyses revealed that CAP values were significantly associated with BMI (r=0.47, p<0.001), waist circumference (r=0.44, p<0.001), hip circumference (r=0.33, p<0.001), AST (r=0.20, p=0.02), ALT (r=0.22, p=0.01), triglycerides (r=0.25, p=0.006), and total cholesterol (r=0.19, p=0.02). BMI was the only independent predictor of CAP in multivariable stepwise linear regression analyses (beta=0.46, t=5.5, p<0.001).

ALT levels in patients with TE-defined fibrosis and steatosis

Since liver enzymes are commonly used to screen for NAFLD in the clinical setting (5,6), we examine the ALT levels in patients with T2DM in relation to TE-defined hepatic fibrosis and steatosis (Table 2). Compared with patients without F3/4 fibrosis, higher ALT levels (p<0.05) were evident in patients with F3 fibrosis, but not in those with F4 fibrosis (p=0.54). Both patients with mild and severe steatosis had higher ALT levels than those without (both p<0.05). However, ALT values did not differ significantly according to steatosis severity (p=0.69).

DISCUSSION

Increasing evidence indicates that the epidemiology of NAFLD is significantly influenced by ethnicity (15,16). In addition to disease prevalence and risk factors, ethnic-related variables may also play a significant role in designing screening programs. Therefore, we specifically investigated whether TE, which has been previously shown to be clinically useful in different geographical populations (11-14), could serve as a non-invasive screening modality for identifying hepatic fibrosis and steatosis in a high-risk cohort of Turkish patients with T2DM. The main findings of the present study are as follows. First, we have shown

that TE-defined advanced fibrosis (≥F3) and cirrhosis (F4) were present in 16.9% and 8.0% of the study patients, respectively. Second, TE-defined steatosis was found to be extremely common, being identifiable in >94% of our subjects. Notably, all cases of steatosis were moderate-to-severe, with no mild forms being observed. Third, BMI was identified as the only independent predictor of both TE-defined fibrosis and steatosis in multivariable analysis. Finally, ALT levels, the most common laboratory parameter used to screen for NAFLD in the clinical routine, were surprisingly unaltered in patients with TE-defined cirrhosis and did not distinguish between different grades of TE-diagnosed steatosis. Generally, our results confirm that TE is a clinically useful non-invasive screening modality for identifying hepatic involvement in Turkish patients with T2DM.

In compliance with previous studies from Romania (11), Hong Kong (12), Korea (13), and France (14), hepatic fibrosis and steatosis were highly common in Turkish patients with T2DM. We believe that TE could serve as a valuable screening modality in this high-risk group owing to its well-known advantages over traditional ultrasound (e.g., ability to identify fibrosis and even low-grade fat deposition and absence of machine and operator dependency) (22). Owing to the strong association between BMI and both fibrosis and/steatosis observed in our study, TE examinations appear to be especially advisable for patients with T2DM who are overweight/obese. However, the costs of this imaging modality need to be weighed against the expected benefits before considering its implementation on a large scale (9). Since different ethnic groups may differ in terms of lifestyles, calorie consumption, levels of physical activity, and genetic determinants of NAFLD (16), not only the diagnostic accuracy of TE but also its cost-effectiveness may vary according to different geographical areas. Our study did not specifically compare the accuracy of TE and ultrasound for NAFLD screening in patients with T2DM. TE is considered to be more sensitive than ultrasound, which is nonetheless less expensive. Future studies should specifically compare the cost-effectiveness of the two techniques. However, our current data strongly suggest that the simple measurement of liver enzymes is not sufficient to detect liver involvement in the setting of T2DM. Accordingly, not only ALT was not elevated in patients with cirrhosis but also it did not distinguish the severity of TE-identified steatosis.

Our study has some limitations. Owing to ethical reasons, liver biopsy for diagnostic confirmation of TE findings was not performed. Although it may be argued that the prevalence of TE-defined steatosis in our sample is very high (94.3%), we believe that our data are in agreement with the published literature. For example, the study conducted in Hong Kong by Kwok et al. (12) reported a prevalence of 70% for TE-defined steatosis. However, the mean BMI of their patients was markedly lower than that observed in our cohort (26.6 kg/m^2 vs. 33.2±6.6 kg/m^2, respectively). Since the sample size of our current study is relatively small, the generalizability of our findings needs to be confirmed in larger studies. Finally, repeated or serial measurements of LSM and CAP were not available. However, a significant strength of our study is that we were able to minimize the number of patients (n=3) who had unreliable measurements, possibly owing to our use of the XL probe in selected obese patients.

In conclusion, our results obtained in a Turkish population expand previous evidence in different ethnicities supporting the clinical value of LSM and CAP for simultaneous screening of hepatic fibrosis and steatosis in patients with T2DM.

Ethics Committee Approval: Ethics committee approval was received for this study from the Institutional Review Board of Marmara University School of Medicine.

Informed Consent: Written informed consent was obtained from all the patients who participated in the study.

Peer-review: Externally peer review.

Author Contributions: Concept - M.D., O.D., Y.Y.; Design - Y.Y., O.D.; Supervision - Y.Y.; Resources - Y.Y.; Materials - M.D., O.D.; Data Collection and/or Processing - M.D., Y.Y.; Analysis and/or Interpretation - M.D., O.D., Y.Y.; Literature Search - Y.Y., M.D.; Critical Review - M.D., O.D., Y.Y.

Conflict of Interest: The authors declare that they have no conflicts of interest.

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