NON-INVASIVE ASSESSMENT OF LIVER STEATOSIS AND FIBROSIS USING TRANSIENT ELASTOGRAPHY AND CONTROLLED ATTENUATION PARAMETER IN TYPE 2 DIABETES PATIENTS

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Abstract

Context. Nonalcoholic fatty liver disease is common in type 2 diabetes mellitus patients, being difficult to diagnose.

Objective. To find a correlation between elastographic parameters and lab results, for facilitating the diagnosis of nonalcoholic fatty liver disease.

Design. This is a cross sectional study, conducted at the Departments of Diabetes, Nutrition and Metabolic Diseases, and Gastroenterology and Hepatology, of the Clinical Emergency Hospital “Pius Brinzeu” Timisoara.

Subjects and Methods. We included 190 type 2 diabetes mellitus patients, collected data regarding medical history, clinical and biological features and applied the Alcohol Use Disorders Identification Test. We excluded patients with other causes of liver disease. Liver steatosis and fibrosis were evaluated through transient elastography, yielding two parameters: liver stiffness as an indicator of liver fibrosis stage, expressed in kPa, and liver steatosis stage, assessed by controlled attenuation parameter, expressed in dB/m. Data were analyzed using SPSS 15.

Results. The analyzed group comprised 113 patients. Elastographic measurements showed that 93.8% of the patients had steatosis (controlled attenuation parameter ≥ 232.5 dB/m) and 70.8% severe steatosis (controlled attenuation parameter ≥ 290 dB/m). Severe steatosis was more common in women (75.7%) than in men (68.1%) ($p < 0.0001$). From the patients with steatosis, 47.2% had liver stiffness values suggestive for fibrosis and 19.8% for cirrhosis. Most patients with steatosis and severe fibrosis were obese (66.7%). Triglycerides/HDLc ratio > 4 correlated with hepatic steatosis ($p = 0.04$), being more common in patients with severe fibrosis/cirrhosis (58.3%) than in those with absent or mild fibrosis (36.2%).

Conclusions. Our study found a clear correlation between type 2 diabetes mellitus and the presence of liver steatosis. It correlates with body mass index, waist circumference (in men) and triglycerides/HDLc ratio. Controlled attenuation parameter is a useful noninvasive method for detection and quantification of liver steatosis.

Key words: type 2 diabetes mellitus, nonalcoholic fatty liver disease, Transient Elastography, Controlled Attenuation Parameter, triglycerides/HDLc ratio.

INTRODUCTION

Recently published studies showed a worrying increase in the prevalence of type 2 diabetes mellitus (T2DM) and of the associated comorbidities, such as obesity and other components of the metabolic syndrome. In parallel, there is an increase in the prevalence of nonalcoholic fatty liver disease (NAFLD), which tends to become the most common liver disease in the western world (1). It is considered to affect 20-30% of the general population (2). The prevalence is higher in patients with T2DM (70%) and in those with morbid obesity (90%) (3). The prevalence of nonalcoholic steatohepatitis (NASH) varies between 25 and 55% in patients with morbid obesity, diabetes and metabolic syndrome (4).

NAFLD occurs more frequently in patients with T2DM, regardless of the duration of the disease or the use of antihyperglycemic therapy, and it is closely related to abdominal obesity, high blood pressure, insulin resistance and dyslipidemia.

NASH, where steatosis is associated with inflammation, can progress in time to the advanced stages of liver fibrosis and cirrhosis, situation that occurs in 10-25% of the cases (5-7). An accurate diagnosis of NAFLD requires liver biopsy. This is an invasive investigation, usually not performed due to patient's refusal, on the one hand, and due to the need of a highly specialized medical team, on the other hand. That is why it is necessary to find a simpler, noninvasive, clinical and biological algorithm that allows an early diagnosis.

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The main purpose of this study was to find a correlation between elastographic parameters and lab results, in order to facilitate the early diagnosis of NAFLD and to prevent its progression towards severe stages. We also aimed to highlight a possible correlation between NAFLD and TG/HDLc ratio (known as a marker of insulin resistance).

PATIENTS AND METHODS

The present cross sectional study was conducted at the Department of Diabetes, Nutrition and Metabolic Diseases of the Clinical Emergency Hospital “Pius Brinzeu” Timisoara in the interval October-December 2016, in collaboration with the Department of Gastroenterology and Hepatology from the same hospital.

Patients

We randomly included 190 patients with T2DM showing up for their periodic diabetologic check-up (we included every third patient showing up, in order to avoid potential bias related to the inclusion of members of the same family, and to prevent the overload of the schedule). The County Emergency Hospital Ethics Committee (Board of Human Studies) approved the protocol, and every patient provided written informed consent before enrolment. Data regarding medical history, clinical and biological features were collected for all the subjects. History taking included demographic data (gender, date of birth) and medical history (date of diabetes onset, antihyperglycemic therapy and other known diseases such as liver disease, dyslipidemia, high blood pressure). For all patients we performed a physical examination that included anthropometric measurements (height, weight, waist circumference), we calculated the body mass index (BMI) using the formula $BMI = \text{weight}/\text{height}^2$, and we applied a questionnaire on alcohol consumption (the Alcohol Use Disorders Identification Test – AUDIT-C), calculating the score.

We excluded from the analysis patients with increased alcohol consumption, meaning an AUDIT-C score higher than 8 (8), and patients with known liver disease due to other causes (viral hepatitis, autoimmune diseases, etc.).

In all included patients the following lab parameters were assessed: serum total cholesterol, triglycerides – TG –, HDLc, LDLc, aminotransferases (AST, ALT), HbA1c, and the TG/HDLc ratio was calculated. Because recent studies showed that an increased TG/HDLc ratio is associated with an increased

cardiovascular risk and with insulin resistance, we tried to find a correlation between NAFLD and TG/HDLc ratio (9-12).

Elastographic assessment

In all patients, liver steatosis and fibrosis were evaluated first by abdominal ultrasound examination and then by Transient Elastography (TE – FibroScan, EchoSense), in the same session, yielding two parameters: the liver stiffness (LS) as an indicator of the liver fibrosis stage, expressed in kPa, and liver steatosis severity assessed by the controlled attenuation parameter (CAP), expressed in dB/m.

All ultrasound examinations and elastographic measurements were performed in fasting patients. For elastography, the patients were examined in supine position with the right arm in abduction. The examinations were performed by two investigators (SN, RM), highly qualified in elastography.

Valid values for TE examination were defined as the median of 10 measurements with IQR/median <30%. For LS and CAP we used either the M or the XL probe, depending on the fat tissue layer of the patient: M probe in patients with a skin-to-liver capsule distance <25 mm, and XL probe in patients with the distance ≥25 mm. Patients for whom we did not achieve valid results were excluded from the analysis.

All included patients were classified according to liver fibrosis severity assessed by TE in 4 stages, as follows (13): stage F0/F1 (absent/mild fibrosis), defined by LS <6.2 kPa; stage F2 (significant fibrosis), with LS ranging from 6.2 to 8.19 kPa; stage F3 (severe fibrosis), with LS ranging from 8.2 to 9.49 kPa; and stage F4 (cirrhosis), as indicated by LS ≥9.5 kPa.

In addition, all patients were classified regarding the severity of steatosis, based on CAP values, in 4 stages, as well (14): stage S0 (absence of steatosis) with CAP <232.5 dB/m; stage S1 (mild steatosis), with CAP ranging from 232.5 to 254.99 dB/m; stage S2 (moderate steatosis), with CAP ranging from 255 to 289.99 dB/m; and stage S3 (severe steatosis) with CAP ≥290 dB/m.

Statistical analysis

The collected data were analyzed using SPSS version 15 (SPSS, Chicago, IL). We calculated mean ± standard deviation, median range or percentages. The significance of the differences was assessed by the Student's t test, ANOVA and chi-square test. A p-value <0.05 was considered statistically significant. In the case of Pearsons' correlation, a value $r > 0.4$ was considered a good correlation.

RESULTS

From the initially included 190 patients, 77 cases were excluded due to situations that would have influenced the results: 49 patients (25.8%) because of the inability to obtain valid measurements by TE (IQR/median >30% or inability to obtain 10 valid measurements), despite the use of both probes, 18 patients (9.4%) due to the presence of other known liver diseases (chronic hepatitis B or C, autoimmune hepatitis, etc.) and 10 cases (5.2%) because of a harmful alcohol consumption, expressed by an AUDIT-C score >8.

After excluding these subjects, the analyzed group comprised 113 patients. The patients' demographic characteristics and their main clinical and biological data are presented in Table 1. The percentage of overweight patients (BMI ≥ 25 kg/m²) was similar in women and in men (91.3% vs. 84%, $p=0.4$).

Overall prevalence of steatosis

CAP measurements showed that 106 patients (93.8%) had steatosis (defined as CAP ≥ 232.5 dB/m), 66 women and 40 men. It is worth mentioning that 70.8% of the patients had CAP values ≥ 290 dB/m, indicating severe steatosis. Severe steatosis was not statistically significant more common in women (72.4%) than

in men (68.1%). Only 6.2% patients had CAP values indicating the absence of steatosis (Table 2).

Overall prevalence of fibrosis

After performing TE, we observed that about half of the patients (54.9%) had absent or mild fibrosis (LS <6.2 kPa), while 45.1% had some degree of fibrosis; significant fibrosis (F=2) was present in 15.9% of the cases, severe fibrosis (F3) in 10.6%, while LS values indicative of F4 (cirrhosis) were observed in 18.6% of the cases.

Correlation between steatosis and fibrosis

Only 1 (14.3%) of the patients without steatosis had LS values suggestive of significant fibrosis (7.2 kPa). From the patients with mild steatosis, 1 (20%) had LS values suggestive of cirrhosis (stage F4). From the patients with moderate steatosis, 38.1% had significant fibrosis (F ≥ 2). In the group of patients with severe steatosis, more than half (51.2%) had some degree of fibrosis.

Analysis of the patients with steatosis

By analyzing the presence of fibrosis in the 106 patients with hepatic steatosis, we found that 47.2% had different stages of fibrosis, 19.8% being classified

Table 1. Patients' characteristics

Feature	Result
Number of patients	113
Gender distribution	
Women, n (%)	69 (61)
Men, n (%)	44 (38.9)
Age	
Mean (years)	60.4 $\pm$ 8.9
Minimum (years)	29
Maximum (years)	81
Mean BMI (kg/m ²)	31.9 $\pm$ 5.8
Underweight patients (BMI <18.5 kg/m ²) (%)	0
Normal weight patients (BMI = 18.5-24.9 kg/m ²) (%)	11.5
Overweight patients (BMI = 25-30 kg/m ²) (%)	27.5
Obese patients (BMI >30 kg/m ²) (%)	61
Average waist circumference, in women (cm)	101.8 $\pm$ 8.6
Average waist circumference, in men (cm)	116.3 $\pm$ 10.5
Patients with triglycerides >150 mg/dL (%)	42.4

Legend: BMI = body mass index

Table 2. Distribution of the patients according to steatosis severity

Steatosis severity	S0	S1	S2	S3
Number	7	5	21	80
Overall prevalence	6.2%	4.4%	18.6%	70.8%
Prevalence in women	4.4%	5.8%	17.4%	72.4%
Prevalence in men	9.1%	2.2%	20.5%	68.2%
p value (chi-square test)	NS	0.01	NS	NS

Legend: NS = not statistically significant

as cirrhosis. The prevalence of cirrhosis was similar in women (24.2%) and in men (12.5%) ($p=0.9$) (Table 3).

The subgroup of patients with severe steatosis (S3) consisted of 80 subjects, 50 women and 30 men. Analyzing this subgroup regarding fibrosis, we observed that 51.2% had different stages of liver fibrosis, cirrhosis (F4) being present in 22.5% of the patients, a prevalence of 30% in women and of 10% in men (10%) ($p>0.05$) (Table 4).

Regarding the role of obesity, we analyzed the weight status of the 106 patients with liver steatosis, depending on the stage of fibrosis. We noticed that among patients with significant liver fibrosis (F2-F4), only 8.5% had a normal weight, 26.5% being overweight and 65% obese. From the patients with steatosis and severe fibrosis (F4), we observed that 9.5% had a normal weight, 23.8% overweight, and 66.7% obesity (Table 5).

In addition, we analyzed the correlation between steatosis and waist circumference, as a marker of excessive weight and insulin resistance. We found significant differences between the groups of patients with different degrees of steatosis in terms of BMI, in both genders ($p=0.01$), and of abdominal circumference, in men ($p=0.0002$). Mean waist circumference was significantly higher in men with severe steatosis than in those without ($p=0.0002$) (Table 6).

TG/HDL ratio

We analyzed the TG/HDLc ratio in patients with steatosis and found that a value >4 correlated with hepatic steatosis ($p=0.04$), being present in 36.2% of the analyzed patients, significantly more frequent in women ($p=0.02$) (Table 7). Increased TG/HDLc ratio was non-significantly more common in patients with severe fibrosis/cirrhosis (58.3%) than in those with absent or mild fibrosis (36.2%) ($p=0.4$).

Patients with a TG/HDLc >4 had a significantly lower mean age ($p=0.03$), a significantly higher mean BMI ($p=0.04$), a significantly higher waist circumference, both in men ($p=0.05$) and in women ($p=0.01$), significantly higher average scores of liver fibrosis and steatosis ($p=0.02$, and $p=0.04$, respectively), and a poor glycemic control ($p=0.04$) (Table 7).

DISCUSSION

Liver steatosis in patients with minimal or no alcohol consumption is a medical condition often associated with insulin resistance, namely obesity, diabetes, dyslipidemia, and also involves an increased cardiovascular risk. Although often benign, it can evolve in several stages: from simple hepatic steatosis, to NASH, fibrosis and cirrhosis. The exact prevalence is not known, because it is difficult to routinely explore, but the published figures are worrying.

Table 3. Fibrosis in patients with steatosis (106 subjects)

Stage of fibrosis	F0/F1	F2	F3	F4
Number	56	17	12	21
Overall prevalence	52.8%	16.1%	11.3%	19.8%
Prevalence in women	51.6%	15.1%	9.1%	24.2%
Prevalence in men	55%	17.5%	15%	12.5%
p value (chi-square test)	NS	NS	NS	NS

Legend: NS = not statistically significant

Table 4. Fibrosis in patients with severe steatosis (80 subjects)

Stage of fibrosis	F1	F2	F3	F4
Number	39	17	6	18
Overall prevalence	48.8%	21.2%	7.5%	22.5%
Prevalence in women	46%	20%	4%	30%
Prevalence in men	53.3%	23.4%	13.3%	10%
p value (chi-square test)	NS	NS	NS	NS

Legend: NS = not statistically significant

Table 5. Weight status and fibrosis in patients with steatosis (106 subjects)

Weight status	Hepatic fibrosis			
	F0/F1 N=56	F2 N=17	F3 N=12	F4 N=21
Normal weight, n=9	7.1%	11.8%	8.4%	9.5%
Overweight, n=28	21.4%	35.2%	41.6%	23.8%
Obesity, n=69	71.5%	53%	50%	66.7%

Table 6. Anthropometric and biological features of patients with different degrees of steatosis

Feature	S0	S1	S2	S3	p value ANOVA
Number of patients	7	5	21	80	-
Mean age (years)	62.4±10	63.2±1.5	61.4±8.1	59.9±9.3	NS
Mean BMI (kg/m ²)	25.6±3.3	34.5±4.3	29.7±6.8	32.9±5.4	0.01
Mean waist circumference, in women (cm)	97±8.9	110.2±2.9	107.2±13.8	108.2±11.9	NS
Mean waist circumference, in men (cm)	89±5.5	108	99.1±8.2	112.4±10.3	0.0002
Women with waist circumference >80 cm (%)	100	100	91.7	90	0.4*
Men with waist circumference >94 cm (%)	0	100	77.8	93.3	<0.0001*
Mean AST (U/L)	38.4±38.5	25.5±7.6	24.8±14.1	33.4±18.3	NS
Mean ALT (U/L)	35.5±29.5	28.2±10.6	34.5±17.1	47.3±27.4	NS
Patients with AST >40 U/L (%)	14.3	0	9.5	29.7	0.07*
Patients with ALT >40 U/L (%)	14.3	0	28.6	48.6	0.01*
Mean HbA _{1c} (%)	8.4±1.1	6.8±1.9	7.4±1.6	8.1±1.7	NS
Mean total cholesterol (mg/dL)	158.8±42.5	188.4±37.8	189.9±46.2	187.5±48.1	NS
Patients with total cholesterol >200 mg/dL (%)	14.3	40	33.3	36.9	NS
Mean triglycerides (mg/dL)	197.2±165.3	123±27.9	152.6±68.9	183.7±96	NS
Median value of triglycerides (mg/dL)	103	140	139	160	NS
Patients with triglycerides >150 mg/dL (%)	28.6	0	38.1	52.7	0.06
Mean HDLc (mg/dL)	28.3±2	51.4±15.3	52.7±19.4	43.9±14.8	NS
Men with HDLc <40 mg/dL (%)	50	100	33.3	43.3	NS
Women with HDLc <50 mg/dL (%)	33.3	50	40	58	NS
TG/HDLc (mean)	24.4±37.8	2.6±1.1	3.3±1.9	4.9±3.8	0.02
TG/HDLc (median)	3.55	2.17	2.54	3.87	<0.0001
Patients with TG/HDLc >4 (%)	14.3	20	28.6	41.2	0.06
Mean LDLc (mg/dL)	69.4±51.4	121±35.9	105.5±21.3	109.1±37.8	NS
Patients with LDLc >70 mg/dL (%)	28.5	100	76.2	70	NS

Legend: BMI=body mass index, AST=aspartate aminotransferase, ALT=alanine aminotransferase, HbA_{1c}=glycated hemoglobin, TG=triglycerides, LDLc=low-density lipoprotein cholesterol, HDLc=high-density lipoprotein cholesterol, NS=not statistically significant, *=p value obtained by chi-square test for trend.

Table 7. Anthropometric, biological and imaging parameters in patients with TG/HDLc <4 vs. TG/HDLc >4

Parameter	TG/HDLc <4	TG/HDLc >4	p value (Student's t-test)
n	55 (56%)	41 (42.7%)	
Women	38 (69%)	22 (53.6%)	0.3*
Men	17 (31%)	19 (46.4%)	0.5*
Mean age (years)	62±8.4	58.1±9.1	0.03
Mean BMI (kg/m ²)	31.4±5.7	33.8±6.1	0.04
Mean waist circumference, women (cm)	106.2±11.5	109.2±10.7	0.01
Mean waist circumference, men (cm)	104.6±11.6	115.7±12.8	0.05
Mean fibrosis score (kPa)	6.6 ±3.4	9.8±8.7	0.02
Mean CAP score (Db/m)	315.1±50.3	334.7±45.3	0.04
Mean HbA _{1c} (%)	7.6±1.5	8.4±1.9	NS
Mean total cholesterol (mg/dL)	186.5±48.3	187.2±48.8	NS
Mean LDLc (mg/dL)	109.9±40	104.2±34.7	NS

Legend: BMI=body mass index, HbA_{1c}=glycated hemoglobin, TG=triglycerides, LDLc=low-density lipoprotein cholesterol, HDLc=high-density lipoprotein cholesterol, NS=not statistically significant, *=p value obtained by chi-square test.

Because NAFLD is a disease with an increasing prevalence and because it implies the risk of developing end-stage liver disease, several studies tried to find algorithms for early diagnosis. Until now, there is no clear diagnostic tool, except for liver biopsy.

In the diagnostic algorithm of NAFLD, the first step is to evidence and to quantify the steatosis, which can be done by abdominal ultrasound, computer tomography (CT), magnetic resonance imaging (MRI), and, more recently by CAP (15). According to the European

guidelines, abdominal ultrasound is the preferred first line method, due to its availability, non-invasiveness and low price, even if its sensitivity is lower in mild steatosis (15). The ultrasound aspect of bright liver with or without posterior attenuation has 64-91% sensitivity, and 93-97% specificity for diagnosing steatosis (16). According to the same authors, in moderate steatosis (affecting more than 30% of the hepatocytes), abdominal US sensitivity increases to 91% (16).

CT and particularly MRI, even if more precise,

are not recommended in clinical practice for steatosis assessment due to their price (MRI) and side-effects (irradiation for CT) (15).

CAP is the “new kid on the block” to assess the severity of steatosis. It is integrated into the newer models of FibroScan devices and it measures the total attenuation of US waves that pass through the liver during TE measurements, being expressed in dB/m, with a range of 100-400 dB/m. A meta-analysis (14) calculated the best cut-off values to discriminate between various degrees of steatosis, considering liver biopsy as the reference method: S1 ($\geq 10\%$ of hepatocytes) – 232.5 dB/m; S2 ($\geq 33\%$ of hepatocytes) – 255 dB/m; S3 ($\geq 66\%$ of hepatocytes) – 290 dB/m (14). The same cut-offs were used in our study.

According to CAP values, liver steatosis was detected in a very high number of patients (93.8%) with T2DM in our cohort, 70.8% of them indicating severe steatosis. Published data suggest that CAP is a good and objective method for assessing steatosis (17), with a sensitivity of 80-85% (18) or higher (19).

NAFLD includes two pathologically distinct conditions with different prognoses: non-alcoholic fatty liver (NAFL) and NASH; the latter covers a wide spectrum of disease severity, including fibrosis, cirrhosis and hepatocellular carcinoma (15). Imaging methods, such as abdominal ultrasound, CT, MRI and CAP, cannot differentiate between NAFL and NASH, a very important step in assessing prognosis, since NAFL is a benign condition. The gold standard method to diagnose NASH is liver biopsy, but it is poorly accepted by patients due to its invasiveness. Non-invasive evaluation methods used to assess NAFLD are either biologic [NAFLD fibrosis score, FIB-4, Enhanced Liver Fibrosis (ELF) score FibroTest®] or elastographic (TE, FibroScan EchoSense) (15). According to the European guidelines, both biologic and elastographic methods are useful to confirm severe fibrosis and cirrhosis. In a recently published study in which LS values obtained by means of TE were compared to liver biopsy, the following cut-offs to predict the severity of fibrosis with a sensitivity $>90\%$ were obtained: 6.2 kPa for $\geq F2$, 8.2 kPa for $\geq F3$, and 9.5 kPa for F4 (13). The same cut-offs were used in our study.

Very recently, two papers suggested that T2DM and obesity can increase artificially the severity of steatosis in CAP evaluation. In a recent published meta-analysis, Karlas *et al.* (20) proposed to decrease the CAP values with 10 dB/m in the presence of NAFLD/NASH, with 10 dB/m for diabetic patients and 4.4 dB/m for every BMI unit above 25 kg/m². This

proposal was commented by Romero-Gomez *et al.* (21), in regard of the validity of CAP in such patients. On the other hand, a recent paper (22) showed that in patients with a CAP value >300 dB/m, the risk of misdiagnosing fibrosis F3-F4 is high. In the light of this new scientific proof, the results of our study must be interpreted with wariness.

The stage of fibrosis is relevant in order to classify the severity of NAFLD. In our study, almost half (47.1%) of the patients with steatosis had LS values suggestive of fibrosis, stage F4 being suspected in 19.8% of the cases, the prevalence being higher in women than in men.

Out of the patients with severe steatosis (S4), more than half (51.2%) had liver fibrosis of different degrees, 22.5% having cirrhosis (F4), this being more prevalent in women than in men. The degree of fibrosis was correlated with BMI, and more than two thirds (66.7%) of the patients with steatosis and cirrhosis (F4) were obese.

Despite obtaining information only from a quite small number of patients, our data are consistent with other recently published results that showed that steatosis is present in an important number of T2DM patients and it was positively correlated with liver fibrosis (23-24).

TG/HDLc ratio is a marker of insulin resistance and it was used in several studies in order to evaluate cardiovascular risk and insulin resistance, a value >4 being considered an important cardiovascular risk factor (9-11) and a marker for insulin resistance (11, 12). In our study group, we investigated the correlation between TG/HDLc ratio and the presence of liver steatosis and fibrosis. We noticed that a TG/HDLc ratio >4 was more common in patients with severe fibrosis/cirrhosis (58.3%) than in those with absent/mild fibrosis (36.2%). We also observed correlations with HbA1c, duration of diabetes, stage of fibrosis or steatosis. Further evaluations and more patients are needed to better integrate this marker in the early diagnosis of NAFLD.

In the present days, T2DM is considered a major risk factor for NAFLD (25-28). These two diseases co-exist due to their common risk factors, and, therefore, one of the major issues of the modern healthcare is the augmented prevalence of both T2DM and NAFLD, parallel with the epidemic of obesity and insulin resistance.

In conclusion, despite including only a small number of patients (fact that may limit the power of the statistical analysis), our study found a correlation between T2DM and the presence of liver steatosis.

Hepatic steatosis in patients with T2DM is very common and it correlates with BMI, waist circumference (in men) and TG/HDLc ratio. LS in these patients evaluated with TE shows increased values in a significant percent of cases. Systematic evaluation of T2DM patients with regard to the presence of liver steatosis and fibrosis can be proposed as a method for the early diagnosis of pathological liver changes, in order to establish a plan to reverse or to stop the disease progression. Based on the clinical correlates yielded in our study, we suggest that the most at hand measures are represented by TE, CAP and the correlation with TG/HDLc ratio.

Conflict of interest

The authors declare that they have no conflict of interest.

References

- Adams LA, Angulo P, Lindor KD. Nonalcoholic fatty liver disease. *CMAJ* 2005; 172(7):899-905.
- Lazo M, Hernaez R, Eberhardt MS, Bonekamp S, Kamel I, Guallar E, Koteish A, Brancati FL, Clark JM. Prevalence of nonalcoholic fatty liver disease in the United States: the Third National Health and Nutrition Examination Survey, 1988-1994. *Am J Epidemiol* 2013; 178(1):38-45.
- Monjur A. Non-alcoholic fatty liver disease in 2015. *World J Hepatol* 2015; 7(11):1450-1459.
- Clark JM. The epidemiology of nonalcoholic fatty liver disease in adults. *J Clin Gastroenterol* 2006; 40(Suppl 1):S5-S10.
- Ekstedt M, Franzén LE, Mathiesen UL, Thorelius L, Holmqvist M, Bodemar G, Kechagias S. Long-term follow-up of patients with NAFLD and elevated liver enzymes. *Hepatology* 2006; 44(4):865-873.
- Bertot LC, Adams LA. The natural course of non-alcoholic fatty liver disease. *Int J Mol Sci* 2016; 17(5):774-786.
- Caldwell S. Cryptogenic cirrhosis: what are we missing? *Curr Gastroenterol Rep* 2010; 12(1):40-48.
- Bush K, Kivlahan DR, McDonnell MB, Fihn SD, Bradley KA. The AUDIT alcohol consumption questions (AUDIT-C): an effective brief screening test for problem drinking. *Arch Intern Med* 1998; 158(16):1789-1795.
- da Luz PL, Favarato D, Faria-Neto JR Jr, Lemos P, Chagas AC. High ratio of triglycerides to HDL-cholesterol predicts extensive coronary disease. *Clinics (Sao Paulo)* 2008; 63(4):427-432.
- Gaziano JM, Hennekens CH, O'Donnell CJ, Lemos P, Chagas AC. Fasting triglycerides, high density lipoprotein, and risk of myocardial infarction. *Circulation* 1997; 96(8):2520-2525.
- Armato J, Reaven G, Ruby R. Triglyceride/high-density lipoprotein cholesterol concentration ratio identifies accentuated cardio-metabolic risk. *Endocrine Practice* 2015; 21(5):495-500.
- Kim-Dorner SJ, Deuster PA, Zeno SA, Remaley AT, Poth M. Should triglycerides and the triglycerides to high-density lipoprotein cholesterol ratio be used as surrogates for insulin resistance? *Metabolism: clinical and experimental* 2010; 59(2):299-304.
- Cassinotto C, Boursier J, de Lédinghen V, Lebigot J, Lapuyade B, Cales P, Hiriart JB, Michalak S, Bail BL, Cartier V, Mouries A, Oberti F, Fouchard-Hubert I, Vergniol J, Aubé C. Liver stiffness in nonalcoholic fatty liver disease: a comparison of supersonic shear imaging, FibroScan, and ARFI with liver biopsy. *Hepatology* 2016; 63(6):1817-1827.
- Shi KQ, Tang JZ, Zhu XL, Ying L, Li DW, Gao J, Fang YX, Li GL, Song YJ, Deng ZJ, Wu JM, Tang KF. Controlled attenuation parameter for the detection of steatosis severity in chronic liver disease: a meta-analysis of diagnostic accuracy. *J Gastroenterol Hepatol* 2014; 29(6):1149-1158.
- European Association for the Study of Diabetes (EASD); European Association for the Study of Obesity (EASO). EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. *J Hepatol* 2016; 64(6):1388-1402.
- Palmentieri B, de Sio I, La Mura V, Masarone M, Vecchione R, Bruno S, Torella R, Persico M. The role of bright liver echo pattern on ultrasound B-mode examination in the diagnosis of liver steatosis. *Dig Liver Dis* 2006; 38(7):485-489.
- Myers RP, Pollett A, Kirsch R, Pomier-Layrargues G, Beaton M, Levstik M, Duarte-Rojo A, Wong D, Crotty P, Elkashab M. Controlled Attenuation Parameter (CAP). A noninvasive method for the detection of hepatic steatosis based on Transient Elastography. *Liver International* 2012; 32(6):902-910.
- Lupsor-Platon M, Feier D, Stefanescu H, Tamas A, Botan E, Sparchez Z, Maniu A, Badea R. Diagnostic accuracy of controlled attenuation parameter measured by transient elastography for the non-invasive assessment of liver steatosis: a prospective study. *J Gastrointest Liver Dis* 2015; 24(1):35-42.
- Sasso M, Audière S, Kemgang A, Gaouar F, Corpechot C, Chazouillères O, Fournier C, Golsztein O, Prince S, Menu Y, Sandrin L, Miette V. Liver steatosis assessed by controlled attenuation parameter (CAP) measured with the XL probe of the FibroScan: a pilot study assessing diagnostic accuracy. *Ultrasound Med Biol* 2016; 42(1):92-103.
- Karlas T, Petroff D, Sasso M. Individual patient data meta-analysis of controlled attenuation parameter (CAP) technology for assessing steatosis. *J Hepatol* 2017; 66(5):1022-1030.
- Romero-Gómez M, Cortez-Pinto H. Detecting liver fat from viscoelasticity: How good is CAP in clinical practice? The need for universal cut-offs. *J Hepatol* 2017; 66(5):886-887.
- Petta S, Wong VW, Cammà C, Hiriart JB, Wong GL, Vergniol J, Chan AW, Di Marco V, Merrouche W, Chan HL, Marra F, Le-Bail B, Arena U, Craxi A, de Lédinghen V. Serial combination of non-invasive tools improves the diagnostic accuracy of severe liver fibrosis in patients with NAFLD. *Aliment Pharmacol Ther* 2017; 46(6):617-627.
- Kwok R, Choi KC, Wong GL, Zhang Y, Chan HL, Luk AO, Shu SS, Chan AW, Yeung MW, Chan JC, Kong AP, Wong VW. Screening diabetic patients for non-alcoholic fatty liver disease with controlled attenuation parameter and liver stiffness measurements: a prospective cohort study. *Gut* 2016; 65(8):1359-1368.
- Livadariu R, Timofte D, Trifan A, Danila R, Ionescu L, Singeap AM, Ciobanu D. Vitamin D Deficiency, A Noninvasive Marker of Steatohepatitis in Patients with Obesity and Biopsy Proven Nonalcoholic Fatty Liver Disease. *Acta Endocrinol (Buc)* 2018; 14(1):76-84.
- Stepanova M, Aquino R, Alsheddi A, Gupta R, Fang Y, Younossi Z. Clinical predictors of fibrosis in patients with chronic liver disease. *Aliment Pharmacol Ther* 2010; 31(10):1085-1094.
- Suzuki A, Angulo P, Lymp J, St Sauver J, Muto A, Okada T, Lindor K. Chronological development of elevated aminotransferases in a nonalcoholic population. *Hepatology* 2005; 41(1):64-71.
- Doycheva I, Patel N, Peterson M, Loomba R. Prognostic implication of liver histology in patients with nonalcoholic fatty liver disease in diabetes. *J Diabetes Complications* 2013; 27(3):293-300.
- Neuschwander-Tetri BA, Clark JM, Bass NM, Van Natta ML, Unalp-Arida A, Tonascia J, Zein CO, Brunt EM, Kleiner DE, McCullough AJ, Sanyal AJ, Diehl AM, Lavine JE, Chalasani N, Kowdley KV; NASH Clinical Research Network. Clinical, laboratory and histological associations in adults with nonalcoholic fatty liver disease. *Hepatology* 2010; 52(3):913-924.