PREVALENCE OF LIVER FIBROSIS AND STEATOSIS USING VIBRATION-CONTROLLED TRANSIENT ELASTOGRAPHY IN INDIVIDUALS EMPLOYED IN A NORTH-EASTERN ROMANIAN INDUSTRIAL COMPANY

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PREVALENCE OF LIVER FIBROSIS AND STEATOSIS USING VIBRATION-CONTROLLED TRANSIENT ELASTOGRAPHY IN INDIVIDUALS EMPLOYED IN A NORTH-EASTERN ROMANIAN INDUSTRIAL COMPANY (Abstract): Liver cirrhosis is considered the final stage of liver fibrosis progression and the leading cause of worldwide mortality due to liver diseases. Certain liver infections, such as viral hepatitis B (VHB) and C (VHC), alcohol-related liver disease (ALD), and nonalcoholic fatty liver disease (NAFLD) were found to be significant contributors to liver cirrhosis development. The **aim** of our study was to assess the prevalence of liver steatosis and fibrosis in individuals from the industrial field in the Northeastern region of Romania. **Material and methods:** Five hundred and sixty adult asymptomatic participants, all employees of a large bakery enterprise, were selected for this study. After informed consent was obtained, the demographical, clinical, and physiological description was made for each participant. AUDIT-C questionnaire was applied to each participant. VCTE (Fibroscan) was used to measure liver fibrosis, and hepatitis virus B and C presence was screened using rapid blood tests. **Results:** We found that 56.25% of the screened participants were from rural areas, and 65.7% were male. In our study, 54.64% declared that they had a smoking history, and only 1.7% received blood transfusions in the past. Moreover, 61% of the patients had a body mass index above the normal limit, 1.7% of the participants were positive for the presence of HBs antigen, while 2.85%

exhibited HCV antibody-positive titer. During VCTE examinations, for the majority of the patients M probe was used (75.2%). Liver fibrosis was found in 33.2% of the participants, and liver cirrhosis in 8.2% of the cohort. Increased severity of liver steatosis was observed in 33.4% of the patients. **Conclusions:** By screening a cohort of apparently clinically healthy individuals residing in the Northeastern part of Romania and having different socioeconomic profiles, we observed that the frequency of advanced fibrosis is more increased, as compared to available data. **Keywords:** CHRONIC LIVER DISEASE, FIBROSIS, STEATOSIS, VIRAL HEPATITIS, ALCOHOL-RELATED LIVER DISEASE, NONALCOHOLIC FATTY LIVER DISEASE, VIBRATION-CONTROLLED TRANSIENT ELASTOGRAPHY.

Being the main cause of liver-related deaths, cirrhosis is leading to significant global morbidity and mortality and is setting a major financial burden on the healthcare systems (1). Liver cirrhosis is the final stage of liver fibrosis progression, although the prevalence of compensated cirrhosis is underreported since it presents asymptomatically in the early stages (2). Consequently, a significant proportion of people with compensated cirrhosis remains misdiagnosed until the first sign of decompensation, such as ascites, elevated bilirubin levels, variceal hemorrhage, or encephalopathy. The mortality and morbidity rates are much higher than in compensated cirrhosis, with a 1-year case-fatality rate that can surpass 80% in some situations. Nevertheless, this group of patients is typically rapidly linked to medical care (3).

Currently, chronic hepatitis B (HBV) and C (HCV) infections, non-alcoholic fatty liver disease (NAFLD), and alcohol-related liver disease (ALD) are the main causes of liver cirrhosis (4). NAFLD has emerged as the most prevalent cause of chronic liver disease worldwide, affecting about 25% of the adult population worldwide and contributing to increased global morbidity and mortality (5). This is due to the rapidly shifting landscape of the liver cirrhosis etiology as a result of effective antiviral treatments and the successful implementation of vaccination programs for viral hepatitis (6). Moreover, NAFLD is also a prominent cause of liver transplantation and a rising risk factor for hepatocellular carcinoma (HCC). Simple steatosis is regarded as the "benign form" of NAFLD, while nonalcoholic steatohepatitis (NASH), the "progressive form" with various histological features, is linked to the development of liver fibrosis, including cirrhosis and eventually HCC (5). On the other hand, alcoholism continues to be a serious health issue, with 75 million people globally having an alcohol use disorder and a high risk of ALD. ALD is a catch-all phrase that includes the alcoholic fatty liver, alcoholic hepatitis (AH), and liver cirrhosis; nevertheless, data on prevalence rates and clinical and pathological characteristics of patients with early illness are hard to come by (7). By lowering the prevalence of liver cirrhosis and associated complications, such as liver failure, HCC, and death, early diagnosis of chronic liver illnesses may improve patient outcomes while also lessening the burden of the condition. This strategy must employ simple, readily available, and economically feasible noninvasive techniques for the measurement of liver fibrosis in vast groups of asymptomatic people in order to be effective. The aspartate aminotransferase [AST]-to-platelet ratio index [APRI], the fibrosis-4 index

[FIB-4], the body mass index-AST/ALT ratio-diabetes score (BARD), the Forns score, and the NAFLD score (NAS) are a few serological markers that potentially predict future development of cirrhosis and advanced liver disease in the general population (8).

Over the years, guidelines for fibrosis evaluation have consistently supported vibration-controlled transient elastography (VCTE) as the best non-invasive approach, particularly in the case of chronic viral hepatitis. In summary, this method uses pulse-echo ultrasonic recordings to quantify the speed of a mechanically generated shear wave in a significantly larger hepatic parenchyma, with the propagation velocity being closely correlated with hepatic stiffness. This technique is frequently used in clinical settings around the world since it is quick, simple, painless, and has significant repeatability and reproducibility (9). Additionally, in recent years, the inclusion of CAP in FibroScan® (Echosens, Paris, France) equipment, which reflects the liver's fat impedance, has enabled the simultaneous evaluation of hepatic fibrosis and steatosis. Since CAP measures are a quantitative method with significant sensitivity and specificity, they enable us to detect less severe degrees of steatosis than US (10). Herein, we aimed to assess the prevalence of liver steatosis and fibrosis in individuals from the industrial field in Northeastern region of Romania.

MATERIAL AND METHODS

This study population consisted of apparently healthy individuals residing in two neighboring counties from North-Eastern Romania, all employees in the bakery industry. This geographic area is known at risk for a number of reasons, including increased exposure to risk factors like tobacco use, consumption of calorie-dense and high-fat foods, heavy alcohol intake, physical inactivity, high body mass index (BMI $\geq$ 25 kg/m^2), and restricted access to healthcare services. All participants in this study were above the age of 18, had no prior history of chronic liver disease, and gave their informed consent by signing the study's consent form. Moreover, the individuals included in our study were workers at S.C. MOPAN SUCEAVA S.A., a large bread and bakery factory, and were evaluated due to an association between Institute of Gastroenterology and Hepatology, and this company through Platform for Interdisciplinary Research, "Grigore T. Popa" University of Medicine and Pharmacy. All patients who had a history of chronic viral hepatitis HBV/HCV/ hepatitis D virus (HDV) infection or other causes of chronic liver diseases, pregnant women, individuals with cardiac pacemakers, cancer or end-stage renal diseases, or had unreliable or failed VCTE measurements were excluded. Subjects examined by VCTE were further assessed through rapid diagnostic tests to detect HBs antigen (HBsAg) and anti-HCV antibodies (HCVA): HBV (Wama Immuno-Rapid HBV®) and HCV (Wama Immuno-Rapid HCV®) and completed the AUDIT-C questionnaire to establish the alcohol consumption. The threshold that rules in subjects with excessive alcohol intake usually is 20 g per day in women, and 30 g per day in men according to recent scientific recommendations (11). Using a height meter and a weight scale, measurements of height and weight were recorded. The midpoint between the lower border of the rib cage and the iliac crest was used to calculate waist circumference using a height meter. As proxies for adiposity, the body mass

index (BMI) and waist circumference were determined. The World Health Organization established cut-off values for overweight (25 kg/m^2) and obesity (>30 kg/m^2). Abdominal obesity was defined as having a waist circumference of 80 cm or more for women and 94 cm or more for males. This measurement is frequently used as a proxy for measuring visceral adiposity (12). The Ethics Committee of our Institute approved the study activities, which were conducted in conformity with the Declaration of Helsinki's guiding principles. Before the examination, all subjects gave their free and informed consent.

The FibroScan 520 Compact device (Echosens, Paris, France) was used for the VCTE and CAP procedures. It is outfitted with the M (regular probe - transducer frequency 3.5 MHz) and XL (transducer frequency 2.5 MHz) probes. An experienced doctor who has performed more than 300 examinations conducted each one in accordance with the procedure's instructions (13). The liver fibrosis stages were identified by the following cut-off values: mild fibrosis 5.6 kPa, considerable fibrosis 8.0 kPa, advanced fibrosis 9.6 kPa, and cirrhosis 13 kPa; LSM results were expressed in kilopascals (kPa) ranging from 1.5 to 75 kPa; The degree of steatosis was separated by the following cut-offs: mild steatosis 274 dB/m, (moderate steatosis) 290 dB/m, and severe steatosis 302 dB/m. CAP was expressed in decibels/m, with a range of 100 to 400 dB/m (9).

The *SPSS* software (version 22.0, IBM SPP Inc., Chicago, IL, USA) was used to perform the statistical analyses. Quantitative variables were expressed as means ± standard deviation (SD), whilst qualitative data were expressed as numbers and/or percentages (%). For distribution analysis, the Kolmogorov-Smirnov test was applied. The student's t-test, Mann-Whitney U test, or chi-square test were deemed suitable for comparing group variables. The Pearson correlation coefficient (r) was used to determine the relationship between two variables. Statistical significance was defined as two-tailed p-values 0.05.

RESULTS

In total, 560 individuals had been screened when the call for participants came to an end. Their median age was 48.14 ± 7.9 years, and 65.7% of them were male. Most of them were from rural area (56.25%). Professional school was the most common achieved educational level (43.9%), followed by high school and junior high school levels (30% and 16.8%, respectively). Only 9.3% of the population had the elementary school level in this regard. 306 (54.64%) of respondents said they had ever smoked cigarettes. 7.7% of the population said they have ever received blood transfusions. Also, among them 43.58% were positive for AgHBs and anti-HCV antibodies. On the other hand, 159 (28.4%) of patients reported consuming alcohol. In this cohort the prevalence rate of type 2 diabetes mellitus was 20.4%. 293 patients (52.3%) had high blood pressure, and 47.1% of individuals had dyslipidemia. Regarding body weight, our participant population was distributed as follows: six individuals (1.1%) were underweighted, 212 participants (37.9%) were normal weight, 207 participants (36.9%) were overweight, and 135 participants (24.1%) were obese. The mean BMI value for our cohort was 27.09 ± 3.12 kg/m^2. Also, 23 (4.1%) patients were AgHbs positive, and 16 (2.85%) detectable HCV antibody titer (tab. I).

TABLE I.
Baseline characteristics of studied population

	Overall Cohort N = 560
Age (years)	48.14 ± 7.9
Males, n (%)	368 (65.7)
Weight	79.26 ± 16.11
Height	169.48 ± 9.83
Body mass index (kg/m^2)	27.09 ± 3.12
Underweight, n (%)	6 (1.1)
Lean subjects, n (%)	212 (37.9)
Overweight, n (%)	207 (36.9)
Obesity, n (%)	135 (24.1)
Hypertension, n (%)	293 (52.3)
Dyslipidemia, n (%)	264 (47.1)
Alcohol intake, n (%)	159 (28.4)
HBV, n (%)	23 (4.1%)
HCV, n (%)	16 (2.85%)
History of type 2 diabetes mellitus, n (%)	114 (20.4)
Smoking history, n (%)	306 (54.64)
Blood transfusion, n (%)	43 (7.7)
Education, n (%)	
Elementary school	52 (9.3)
Junior high school	94 (16.8)
High school	168 (30)
Professional school	246 (43.9)

Regarding VCTE examinations, M-probe was utilized in 421 (75.2%) participants whereas a XL probe was employed in 139 (24.8%) subjects. Eight people failed the measurement, or 1.42% of the population under the study. The cut-offs used for liver fibrosis were as follows: F0, when LSM < 5.6 kPa, F1, when LSM = 5.6 – 7.9 kPa, F2, when LSM = 8 – 9.5 kPa, F3, when LSM = 9.7 – 12.9 kPa, and F4, when LSM ≥ 13 kPa. According to fibrosis stage, most of the patients had F2 level of liver fibrosis (33.2%). 161 (28.8%) individuals have no fibrosis, and 46 (8.2%) of patients have cirrhosis. The mean value of LSM was 7.2 ± 5.03 kPa. The following were the hepatic steatosis cut-off values: S0 - < 274 dB/m, S1 – 274 – 289 dB/m, S2 – 290 – 301 dB/m, S3 - ≥ 302 dB/m. Regarding steatosis degree, 33.4% of subjects have severe steatosis, with mean CAP value of 278.35 ± 67.82 dB/m (tab. II).

TABLE II.
Characteristics of VCTE examinations

Characteristics	**Mean ± SD or number (%)**
Probe size	
M	421 (75.2)
XL	139 (24.8)
Steatosis degree (dB/m)	
CAP < 274 (dB/m)	122 (21.8)
CAP ≥ 274 (dB/m)	148 (26.4)
CAP ≥ 290 (dB/m)	103 (18.4)
CAP ≥ 302 (dB/m)	187 (33.4)
CAP (mean ± SD) (dB/m)	278.35 ± 67.82
Fibrosis stage (kPa)	
LSM < 5.6 kPa	161 (28.8)
LSM ≥ 5.6 kPa	102 (18.2)
LSM ≥ 8 kPa	186 (33.2)
LSM ≥ 9.6 kPa	65 (11.6)
LSM ≥ 13 kPa	46 (8.2)
LSM (mean ± SD) (kPa)	7.2 ± 5.03

History of alcohol consumption, and presence of chronic viral hepatitis (HBV or HCV) were linked with risk for developing severe liver fibrosis (≥ F3) (r = 0.27, p = 0.04) and (r = 0.36, p < 0.001) (fig. 1). High values of BMI and the presence of type 2 diabetes mellitus were correlated with increased CAP values among our cohort (r = 0.17, p = 0.021) and (r = 0.27, p = 0.03) (fig. 2).

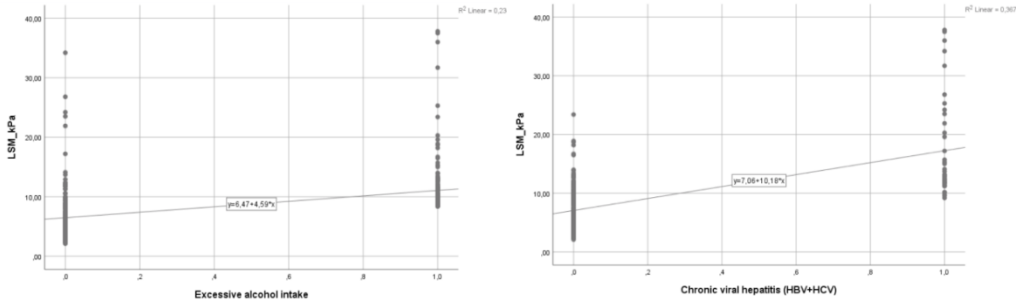

Fig. 1. Correlation between LSM and (A) excessive alcohol intake, (B) chronic viral hepatitis

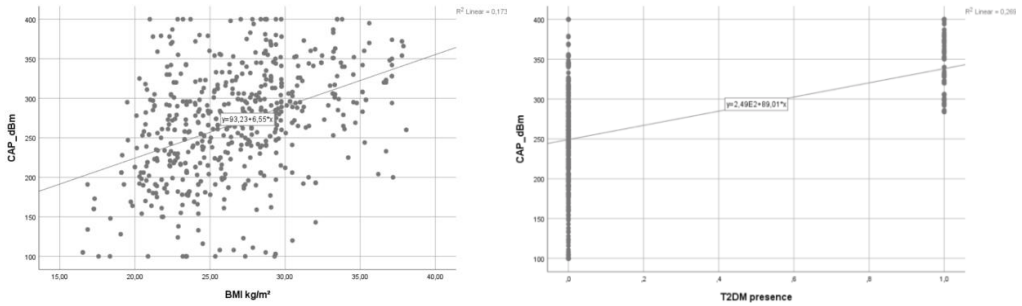

Fig. 2. Correlation between CAP and (A) BMI values, (B) presence of type 2 diabetes mellitus

DISCUSSION

There is a significant financial burden on healthcare systems as a result of liver cirrhosis, which ranks as the 7th most common cause of high disability-adjusted life years and the 11th most common cause of mortality worldwide. The most recent epidemiological research indicate that Europe appears to have the highest global burden of liver disease, and alarming predictions regarding an increase in cases over the next several years are being made (14). The World Health Organization most recent statistics from 2018 show that 8,763 people died in Romania from liver disease, or 3.75% of all deaths. Romania is ranked 47th in the world by age-adjusted death rate, which is 26.90 per 100,000 people. According to data from the Romanian National Institute for Public Health, liver cirrhosis accounted for 34.6 deaths per 100.000 people in 2019 and was the primary cause of mortality in digestive illnesses. With more than 20 deaths per 100,000 people, Lithuania and Hungary both had high mortality rates, at half in comparison with Romania (15).

The analyses of data subsets deriving

from the National Health and Nutrition Examination Survey (NHANES) 2017-2018 revealed that NAFLD had a prevalence of 58.5% and that US fatty liver index (US-FLI) is a reliable scoring system dedicated to diagnosing patients with fatty liver diseases. However, a drawback is that it requires a complementary calculation of insulin levels, for which the odd ratio (OR) was 4.97 in contrast with the indexes for hepatic steatosis (HSI), ORs = 21.2, and fatty liver (FLI) ORs = 18.6 (16).

Another study brought forward both positive and non-significant correlations following CAP and LSM through VCTE assessment of serum uric acid (UA) and marijuana use, whilst no relationships were identified in cases of urinary metabolite concentrations of di-2-ethylhexyl phthalate (DEHP) and vitamin D deficiency. Precisely, Duan *et al.* (17) further emphasize an overall OR of 2.097 and 2.983 and consistence in a sex-matched subgroup, whereas Du *et al.* (18) was able to highlight a low prevalence of steatosis is past ($p = 0.184$) and current users ($p = 0.048$). Recently, Shen *et al.* (19) successfully advanced weight-adjusted-waist index (WWI) to predict hepatic steatosis, WWI suggesting a strong association with hepatic steatosis ($p < 0.0001$) and not liver fibrosis ($p = 0.84$), but extrapolating in relation to excessive alcohol consumption ($p = 0.031$) and hypertension ($p = 0.014$) as multivariate linear regression analysis indicate.

On the other hand, Chen *et al.* (20) failed to observe a significant relationship between liver fibrosis and phthalates for LSM ≥ 8 kPa, but rather in the fourth quartile for mono-(2-ethyl-5-carboxypentyl) phthalate and mono (2-ethyl-5-hydroxyhexyl) phthalate of which the ORs were 2.719 ($p = 0.016$) and 2.073 ($p = 0.037$). This argument of a lack of association extends to the research conducted by Ji *et al.* (21) because the serum vitamin D had a linear trend regardless of participants' group allocation (74.26 *vs.* 72.24 nmol/L for which $p = 0.21$), nor discovered through multivariate logistic regression analysis, instead being uncovered a lower liver fibrosis risk for an OR of 0.89 for a deficiency of vitamin D in NAFLD patients.

Complementary investigations in the field advanced the possibility of enhancing the diagnostic accuracy and thus two teams found out that CAP and LSM identified with an area under the receiver operating characteristic curves (AUROCs) of 0.87 and 0.83 of patients with steatosis, fibrosis and 0.93 those with cirrhosis for the following intervals: 0.77 for S ≥ S1, and S2, and 0.70 for S = S3 at Youden cutoff values of 302, 331, and 337 dB/m, and 0.77 for F ≥ F2, 0.80 for F ≥ F3, and 0.89 for F = F4, respectively, with also Youden cutoff values of 8.2, 9.7, and 13.6 kPa compared with 6.5/12.1 kPa to exclude advanced fibrosis (AF) and cirrhosis at negative predictive values of 0.91 and 0.99. Moreover, a positive predictive value (PPV) of 0.71 and 041, LSM may identify patients with AF and cirrhosis (22, 23). Previous studies from Romania, which were published recently found that in a group of medical students, the prevalence of steatosis and advanced fibrosis was 17.4% and 0.7% respectively, in apparently healthy individuals. Instead, among individuals with type 2 diabetes mellitus the prevalence of advanced fibrosis and cirrhosis was 11.7% and 13.6% respectively (24, 25).

Poverty and chronic liver diseases are linked, due to a variety of factors, such as a higher risk of using tobacco products, eating calorie-dense, high-fat foods, being physically inactive, being overweight or obese, and having less access to healthcare. People who lived in poverty have a higher

mortality rate after being diagnosed with a chronic illness in every country. It's been thought of as rather paradoxical because the poor in middle- and high-income countries tend to be more obese than the wealthy. The same risk factors like in some Romania counties were present in Latin America or Asia, particularly in low- and middle-income nations like Mexico, where the average BMI is approximately 27 kg/m^2, 65% of the population is overweight or obese (BMI $\geq$ 25 kg/m^2), and 26% of the population is obese (BMI $\geq$ 30.27 kg/m^2). Cirrhosis will be one of the most preventable diseases causing death worldwide since it is predicted that the number of deaths worldwide from non-communicable diseases in lower-middle income nations would rise from 13 million in 2005 to approximately 19 million in 2030.

Our study had some limitations. First off, as we included apparently healthy individuals and, no histology data on people with advanced liver fibrosis or cirrhosis were taken due to the lack of liver biopsy. Another drawback is that there was no follow-up to assess if the therapies had changed the CAP score or the LSM, and to maintain monitoring of long-term impacts.

CONCLUSIONS

The assessment of advanced fibrosis by VCTE in a population with particular socioeconomic profile indicates a higher frequency of advanced fibrosis than is apparent in the data available from North-Eastern Romania. In this demographic, obesity is regarded as a separate risk factor for advanced fibrosis. To establish preventive interventions in this demographic area, more research is required.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest.

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