Application of non-invasive methods of assessment of steatosis and fibrosis in chronic diffuse liver diseases of various etiologies

Abstract. Background. The aim of the study is to analyze the parameters of liver steatosis and fibrosis based on shear wave elastography (SWE) and steatometry data in patients with chronic diffuse liver diseases, taking into account the etiological factor, and determine the diagnostic accuracy of SWE in the diagnosis of liver fibrosis.

Materials and methods. Three hundred and sixty-four patients with chronic diffuse liver disease aged (48.00 ± 1.84) years were examined, 159 (43.7 %) were male, and 205 (56.3 %) female. The patients were divided into groups: 108 people with non-alcoholic fatty liver disease (NAFLD), 143 with chronic hepatitis C (HCV), 56 with alcoholic liver disease (ALD), and 57 with drug-induced toxic hepatitis. In all patients, SWE and steatometry were performed by Soneus P7 device (Kharkiv, Ukraine) with the liver stiffness and ultrasound attenuation coefficient measurement.

Results. According to SWE data, 270 (74.2 %) patients with chronic liver disease had fibrotic changes in the liver. A significant increase in liver stiffness by 1.9 times (p < 0.05) according to Young's modulus was found in HCV patients and by 1.4 times (p < 0.05) in ALD patients compared to the control group, by 1.7 (p < 0.05) and 1.3 times (p < 0.05), respectively, compared to the group of patients with NAFLD. According to steatometry data, an increase in ultrasound attenuation coefficient by 30.2 % (p < 0.05) in patients with NAFLD, by 27.5 % (p < 0.05) in those with ALD and by 22 % (p < 0.05) in people with toxic hepatitis was found compared to the control group. In patients with liver fibrosis, the median liver stiffness was 6.70 kPa (6.35, 7.56), while in those without liver fibrosis, this parameter was 1.2 times lower (p < 0.01). Histological evaluation of liver samples obtained through percutaneous biopsy in 75 patients with chronic liver disease demonstrated the absence of fibrosis in 14 (18.7 %) cases. According to the results of the ROC analysis, the cut-off value of the liver stiffness determined by SWE was 5.79 kPa, confirming the presence of liver fibrosis in patients with chronic liver disease regardless of etiology (AUC = 0.901, p < 0.001).

Conclusions. The liver stiffness determined by SWE in HCV and ALD patients was higher than in NAFLD patients (p < 0.05), as well as the frequency of F3–4 stages of liver fibrosis (p < 0.05). The threshold value of the liver stiffness for liver fibrosis diagnosis in chronic liver disease regardless of etiology was 5.79 kPa (sensitivity 100.0 %, specificity 85.7 %), which allows the family doctor to form a risk group of patients who needed dynamic monitoring with a further investigation of the etiological factor of liver fibrosis.

Keywords: non-alcoholic fatty liver disease; alcoholic liver disease, chronic hepatitis C; toxic drug-induced hepatitis; liver fibrosis; diagnosis; shear wave elastography

Introduction

Chronic diffuse liver diseases (CDLD) are characterized by a significant prevalence, minimal clinical manifestations in the initial stages of development, and the possibility of pathological process regression [1].

According to epidemiological studies, the incidence of CDLD worldwide varies from 28.01 to 52.34 per 1,000 individuals per year, with a prevalence of 10–40 % in the population [2]. Chronic liver diseases represent a major global health burden and account for approximately 2 million deaths annually worldwide [3]. The main etiologies of chronic liver disease are considered to be viral hepatitis, alcohol-related damage, metabolic disorders, as well as autoimmune and genetic diseases, toxic and cholestatic liver injuries [4–6].
Liver fibrosis has been proven to correlate with liver function and is a major risk factor for the development of hepatocellular carcinoma [4, 5]. Despite the various mechanisms of primary liver damage, the progression of fibrosis has common patterns [4]. It has been established by scientists that liver fibrosis is a reversible process after the treatment of viral infection and eradication of the pathogen [7]. However, reversion occurs too slowly or infrequently to avoid life-threatening complications, especially in advanced fibrosis [4]. Therefore, early detection of liver fibrotic changes would allow reducing the number of complications and mortality among CDLD patients.

Histological examination of the liver using the Metavir scale is the most informative and objective, allowing the separate consideration of both the degree of inflammation and the degree of liver fibrosis development [8]. However, performing a liver biopsy can lead to complications, including intrahepatic bleeding in approximately 2 % of patients, and is generally not recommended in routine clinical practice at the initial stages of examination. Disadvantages of liver biopsy include pain, invasive nature of the procedure, which can increase mortality rates from 0.009 to 0.14 % [9, 10]. Moreover, conventional morphological examination assesses only approximately 50,000th part of the entire liver parenchyma and can stage gross abnormalities such as septal and periporal fibrosis, scar changes, but does not evaluate soft fibrosis, pericellular, perivenular, or periductular fibrosis. Therefore, in recent years, non-invasive methods for liver fibrosis verification have been frequently employed.

Among the instrumental methods for assessing structural changes in the liver, transient and shear wave elastography (SWE) are currently used to determine fibrosis, and the ultrasound attenuation coefficient is used to determine steatosis.

There is a particular issue with patients with NAFLD who are overweight when it comes to transient elastography. Various studies indicate that frequent failures in the application of transient elastography occur due to an increased body mass index (BMI > 30 kg/m²). The presence of a large subcutaneous adipose tissue interferes with the transmission of shock impulses and proper ultrasound tracking, leading to inaccurate assessments of liver stiffness [11].

Despite the abundant literature evidence supporting the use of transient elastography, a recent study comparing instrumental methods for assessing liver fibrosis in 291 NAFLD patients showed that all methods had high diagnostic accuracy, with area under the ROC curves (AUC ≥ 0.84) for severe fibrosis and cirrhosis, and they demonstrated equal effectiveness in diagnosing this endpoint. However, transient elastography had higher diagnostic efficacy than point shear wave elastography for diagnosing significant fibrosis [11].

Furthermore, there are isolated studies reporting the diagnostic accuracy of transient elastography in various pathological conditions. For instance, Leung et al. compared the results of SWE with liver biopsy in chronic hepatitis B and found that SWE had a sensitivity and specificity of 85 and 92 %, respectively, for diagnosing liver fibrosis, and 97 and 93 % for diagnosing cirrhosis [12].

There is only one study supporting the use of point SWE for assessing alcoholic liver fibrosis [13]. Additionally, there is insufficient evidence to recommend the use of point SWE for differentiating between the absence of fibrosis and mild fibrosis (F0–F1) from significant or severe fibrosis and cirrhosis in alcoholic liver disease (ALD) [13].

In addition, Zayadeen et al. concluded in 2022 that shear wave elastography can be used to assess liver fibrosis regardless of the etiology [7]. Therefore, there is currently a limited number of studies evaluating the effectiveness of SWE in different etiologies of CDLD, and unfortunately, the results are still insufficient to provide recommendations.

The purpose of the study is to analyze the parameters of liver steatosis and fibrosis based on SWE and steatometry data in patients with CDLD, taking into account the etiological factor and determine the diagnostic accuracy of SWE in the diagnosis of liver fibrosis.

**Materials and methods**

Three hundred and sixty-four patients with CDLD aged (48.00 ± 1.84) years were examined, 159 (43.7 %) were male, and 205 (56.3 %) were female. The patients were divided into groups: 108 patients with non-alcoholic fatty liver disease (NAFLD), 143 patients with chronic hepatitis C (CHC), 56 patients with alcoholic liver disease (ALD), and 57 patients with toxic drug-induced hepatitis (TDH).

The inclusion criteria for the study were as follows: patients aged 18 to 75 years, provision of informed consent, completion of all necessary diagnostic and therapeutic procedures, and a confirmed diagnosis of NAFLD, CHC, ALD, or TDH.

The exclusion criteria from the study were as follows: patients younger than 18 or older than 75 years, lack of informed consent, presence of comorbid liver pathology, infection with other viruses (except hepatitis C virus), HIV infection, presence of decompensated somatic pathology, oncological diseases, and pregnancy. Additionally, patients with extraportal portal hypertension, congestive hepatopathy, hematological and lymphoproliferative disorders, portal vein and splenic vein thrombosis, Budd-Chiari syndrome, and uncontrolled diabetes were not included in the study.

In all patients, SWE was performed on the Sonus P7 device (Kharkiv, Ukraine) with a convex probe operating at frequencies of 2–5 MHz at a depth of 10–50 mm from the liver capsule. The median value was determined from these measurements, which characterized liver parenchyma stiffness in kilopascals (kPa). Threshold values proposed by Ferraioli G. were used to assess fibrosis stage: F0–1 stage up to 6.5 kPa, F2 stage up to 7.1 kPa, F3 stage up to 8.7 kPa, and F4 stage 10.4 kPa and above according to the Metavir scale.

Steatometry, a real-time quantitative assessment of liver steatosis, was also performed using the same device. Eight measurements of the ultrasound attenuation coefficient (UAC) were taken in different liver segments. The results were interpreted as follows: S0 corresponded to the “normal” degree of steatosis (hepatocyte fat content ranging from 0 to 5.0 %); 1.0 to 2.19 dB/cm, S1 corresponded to mild steatosis (hepatocyte fat content ranging from 5.1 to 33.0 %); 2.20 to 2.29 dB/cm, S2 corresponded to moderate steatosis (hepatocyte fat content ranging from 33.1 to 66.0 %); 2.30 to 2.90 dB/cm, and S3 corresponded to severe steatosis (hepatocyte fat content exceeding 66.0 %); > 2.90 dB/cm.
Histological evaluation of liver biopsies obtained through percutaneous trephine biopsy was performed in 75 patients with CDLD. Percutaneous puncture trephine liver biopsy was carried out under continuous ultrasound guidance, local anesthesia, and with a semi-automatic 16 G Colt Shot needle. Three tissue specimens from the segment VII of the right lobe were taken from each patient using the percutaneous puncture trephine liver biopsy technique.

Statistical analysis was performed using Statistica 10.0 software. For quantitative variables, the median (Me), lower quartile (Q1), and upper quartile (Q3) were calculated. For qualitative data, absolute frequencies (%) were determined. The Mann-Whitney U test and Kruskal-Wallis test were used for comparing distributions of categorical variables. Differences were considered significant at p < 0.05.

To evaluate the diagnostic significance of quantitative features in predicting the occurrence of a particular result calculated using a regression model, the receiver operating characteristic (ROC) curve analysis was applied. The quality of the predictive model obtained through this method was assessed based on the area under the ROC curve (AUC) with a 95% confidence interval (CI) and the level of statistical significance. Threshold values, sensitivity, and specificity of the indicator were also calculated.

Results

Table 1 presents liver steatometry data in examined patients with various etiologies of CDLD. An increase in the ultrasound attenuation coefficient values, as determined by steatometry, was found to be 30.2 % higher (p < 0.05) in patients with NAFLD, 27.5 % higher (p < 0.05) in patients with ALD, and 22 % higher (p < 0.05) in patients with TDH compared to the group of healthy individuals. Additionally, among the examined patients with CHC, lower values of the coefficient were observed (p < 0.05) compared to the NAFLD and ALD groups.

According to the results of shear wave elastography of the liver, the data of which are shown in Table 2, it was found that in patients with CHC and ALD, a significant increase in the stiffness of the liver parenchyma according to the Young’s modulus indicator was established by 1.9 times (p < 0.05) and by 1.4 times (p < 0.05) compared to the group of healthy individuals. At the same time, with metabolic and toxic damage to the liver, a tendency to increase the stiffness of the liver parenchyma was observed (p > 0.05). A similar picture was observed in relation to such an indicator of the SWE, as the speed of propagation of the shear wave.

Fig. 1 and 2 show elastograms of the liver in patients with ALD, CHC and NAFLD. In total, fibrotic changes in the liver were observed in 270 (74.2 %) patients with chronic liver diseases according to the SWE data, with alcoholic and viral etiology showing fibrosis 1.4 times more frequently compared to the group of patients with metabolic origin: 47 (83.9 %) in ALD and 119 (83.3 %) in CHC compared to 64 (59.3 %) in NAFLD (χ² = 16.69, p < 0.01).

The analysis of the frequency of distribution of patients with CDLD of various etiologies according to the stages of fibrotic changes of the liver showed that in patients with
NAFLD, almost half of the examined had SWE indicators that corresponded to 1 stage of fibrosis (F1) according to the Metavir scale, which significantly distinguished this group from patients with CHC ($\chi^2 = 21.90, p < 0.01$), ALD ($\chi^2 = 9.21, p < 0.01$) and TDH ($\chi^2 = 8.31, p < 0.01$). Two times more often among those examined for ALD and CHC, the indicators of SWE corresponded to 2 stages of liver fibrosis ($\chi^2 = 6.42, p = 0.01$ and $\chi^2 = 6.83, p < 0.01$ compared to NAFLD). Young’s modulus values of 8.7 kPa (F3–4) were more often observed in patients with CHC — 56 (39.2 %) and ALD — 19 (33.9 %) compared to the TDH group — 14 (24.5 %) ($\chi^2 = 10.29, p = 0.001$ and $p > 0.05$, respectively), while individuals with NAFLD had no pronounced fibrosis (Table 3).

At the next stage of the work, an analysis of the results of SWE was carried out depending on the presence of liver fibrosis, according to which in patients with fibrotic changes of the liver, the median stiffness of the parenchyma was 6.70 kPa (6.35; 7.56), while in patients without liver fibrosis, this indicator was probably 1.2 times lower, amounting

Table 3 — Distribution of patients with different etiologies of CDLD according to liver fibrosis stages based on the SWE data

<table>
<thead>
<tr>
<th>Fibrosis stage</th>
<th>NAFLD (n = 108)</th>
<th>CHC (n = 143)</th>
<th>ALD (n = 56)</th>
<th>TDH (n = 57)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>F0</td>
<td>44</td>
<td>40.7</td>
<td>24</td>
<td>16.7*</td>
</tr>
<tr>
<td>F1</td>
<td>53</td>
<td>49.1</td>
<td>29</td>
<td>20.3*</td>
</tr>
<tr>
<td>F2</td>
<td>11</td>
<td>10.2</td>
<td>34</td>
<td>23.8*</td>
</tr>
<tr>
<td>F3</td>
<td>0</td>
<td>0</td>
<td>38</td>
<td>26.6*</td>
</tr>
<tr>
<td>F4</td>
<td>0</td>
<td>0</td>
<td>18</td>
<td>12.6*</td>
</tr>
</tbody>
</table>

Notes: * — $p < 0.05$ — probability of difference compared to the NAFLD group; ** — $p < 0.05$ — probability of difference between the CHC and TDH groups.
to 5.59 kPa (5.50; 5.66) — according to the Kruskal-Wallis test, the probability of the difference was lower than 0.01 (Fig. 3).

In order to determine the diagnostic value of the stiffness index of the liver parenchyma according to SWE data for the evaluation of fibrotic transformation, a comparison was made with the results of histological evaluation of liver biopsies of 75 patients with CDLD, of which 14 (18.7 %) patients had no fibrosis according to morphological data.

During the ROC analysis, a good quality of the diagnostic model was established in the evaluation of the stiffness index of the liver parenchyma according to the SWE data: the area under the ROC curve was 0.901 (95% CI 0.893–0.968; p < 0.01) (Fig. 4).

The threshold value of the Young’s modulus for diagnosing liver fibrosis in patients with CDLD was determined to be 5.79 kPa, with a sensitivity of 100.0 % and specificity of 85.7 %.

**Discussion**

The medico-social significance of the diagnosis of CDLD is due to a significant increase in the number of patients with the specified pathology, especially among people of working age, an increase in the percentage of mortality from complications, as well as an increase in the influence of various risk factors (hepatotoxic doses of alcohol, uncontrolled intake of drugs with an undesirable hepatotoxic effect, viral infections, nutritional features, environmental factors, etc.) [3, 14, 15].

It is known that liver fibrosis is the main pathogenetic process in the progression of CDLD [16–19]. Biopsies, as invasive procedures, have limitations, so the current task remains to deepen our understanding of the effectiveness of non-invasive approaches for determining fibrotic changes in the liver in patients with CDLD depending on the etiology of the disease. We found that patients with CHC and ALD had a significant increase in liver parenchymal stiffness based on the Young’s modulus, which was 1.9 times higher (p < 0.05) and 1.4 times higher (p < 0.05), respectively, compared to the group of healthy individuals, and 1.7 times higher (p < 0.05) and 1.3 times higher (p < 0.05), respectively, compared to the group of patients with NAFLD. Metabolic and toxic liver damage showed a tendency towards increased liver parenchymal stiffness (p > 0.05). Similar results of using SWE in patients with CDLD have also been reported by other authors [7, 11].

Considering that in most cases, a family doctor is unable to immediately determine the etiological cause of CDLD and often there is a combination of multiple etiological factors in one patient, it was reasonable to establish a single objective value of liver parenchymal stiffness as an indicator for assessing the presence of liver fibrosis. Therefore, we conducted an ROC analysis, according to which the threshold value of the Young’s modulus based on elastography data, at which a patient can be classified into the liver fibrosis group in CDLD, is 5.79 kPa.

Implementing this diagnostic criterion in the healthcare system allows the family doctor to identify a high-risk group of patients who are recommended for dynamic monitoring with the possibility of further investigations to clarify the etiological factor of structural changes, namely liver fibrosis.

**Conclusions**

1. The obtained results of non-invasive methods for assessing steatosis and fibrosis in CDLD depend on the etiological factor. In CHC and ALD, the average liver parenchymal stiffness measured by elastography is higher compared to NAFLD (p < 0.05), which confirms a higher frequency of registration of liver fibrosis stages F3–4 (39.2 and 33.9 % versus absence, p < 0.05, respectively).

2. An increase in the value of the ultrasound attenuation coefficient according to steatometry data compared to the group of healthy individuals was established by 30.2 % (p < 0.05) in patients with NAFLD, by 27.5 % (p < 0.05) in patients with ALD and by 22 % (p < 0.05) in patients with TDH.

3. With CDLD without taking into account the etiological factor, the stiffness index of the liver parenchyma, determined using SWE, exceeding the value of 5.79 kPa, confirms the presence of liver fibrosis (AUC = 0.901, p < 0.001), which allows forming a risk group already at the primary level of medical care population of Ukraine.

**References**


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У хворих із фіброзними змінами печінки медіана жорсткості із токсичним гепатитом порівняно з групою здорових осіб.

Патологія печінки і жовчовивідної системи / Pathology of Liver and Biliary Excretion System

Резюме.

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Мета дослідження: проаналізувати показники стеатозу та фіброзу печінки за даними звукохвильової еластографії (ЗХЕ) та стеатометрії в пацієнтів із хронічними дифузними захворюваннями печінки незалежно від етіології (AUC = 0,901, p < 0,001). За результатами ROC-аналізу, показник жорсткості паренхими печінки, визначений за допомогою 3ХЕ, що перевищує 5,79 кПа, підтверджує наявність фіброзу печінки в осіб із хронічними дифузними захворюваннями печінки незалежно від етіології (AUC = 0,901, p < 0,001).

Застосування неінвазивних методів оцінки стеатозу та фіброзу при хронічних дифузних захворюваннях печінки різної етіології

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Authors' contribution. Stepanov Yu.M. — concept of research; Didenko V.I. — design of research, data analysis of instrumental studies; Petishko O.P. — statistical processing of material, design of the article; Halinska A.M. — design of the article, translation.

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