Non-invasive fibrosis markers and elastography in diagnosis of fibrosis severity in patients with type 2 diabetes mellitus and non-alcoholic fatty liver disease


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Key words: fibrosis, elastography, type 2 diabetes mellitus, non-alcoholic fatty liver disease.

Objective: to evaluate the possibility of determining the stage of non-alcoholic fatty liver disease (NAFLD) with the help of indirect non-invasive markers of fibrosis and elastography.

Material and methods. The study involved 43 patients with type 2 diabetes mellitus (DM) and manifestation of NAFLD, including 26 (60.5 %) females and 17 (39.5 %) males. All the patients underwent an extensive ultrasound examination of the liver, namely determination of its size below the costal arch, a duplex examination to assess the distribution of hepatic vessels, determine possible blood flow trouble and its type.

B-mode liver ultrasound with real-time elastography on Hitachi Hi Vision Avius apparatus was also performed. The presence and stage of fibrosis were assessed using the Bonacini discriminant score, METAVIR and Ishak scoring systems. A correlation analysis between results of various methods of liver fibrosis stage assessment in patients with type 2 DM and NAFLD was also conducted.

Results. Generally, in patients with type 2 DM and NAFLD, mild fibrosis (F0–F2) was diagnosed. The assessment of liver fibrosis intensity according to sonoeLASTography and other non-invasive methods (Bonacini, Ishak and METAVIR scores) gave comparable results. The results of liver fibrosis stage assessment in patients with type 2 DM and NAFLD according to sonoeLASTography were strongly correlated with the results of Bonacini classification (discriminant) score. The results of the Ishak and METAVIR scores were moderately correlated with the sonoeLASTography data and strongly correlated precisely in advanced stages of hepatic fibrosis.

Conclusions. The results obtained show the importance of criteria for NAFLD assessment in patients with type 2 DM, the need to determine NAFLD and liver fibrosis stages. The combination of ultrasound diagnosis, serum biomarkers and use of diagnostic scales is more informative and appropriate for assessing the liver fibrosis presence in patients with NAFLD, compared with separate use of these methods allowing reducing the frequency of invasive traumatic methods using.

Neihivazni marke r fibrozou ta elastografii v diagnostyce tashkosti fibrozou u xorhiv na cukrovy diabet 2 typu ta nealkogolnujuhrovu xorobu pechniki

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Meta robiti – oznitni molliwistivivizhennia stavdiinealkogolnojuhryvojehorobipechniki (NAXHP) zadopomoyo nepriamim neihivaznymivmarkev fibrozou ta elastografii.

Materiiali ta metodi. Obstekli 43 osobii, ja k xorvi na cukrovy diabet (CD) 2 tiu z proymi NAXHP: 26 (60,5 %) jinok ta 17 (39,5 %) choviniv. Usem patientam zdaiśihii rozshirenne ulypgrazuvkove obstekhenie pecheni, co peredbičalo vizhennia ji riamu jche kraya rebernoy duhii, duptleksne obstekhenie dli oznchennia xodu sudni pechenii, vizhennia mollywiv peresho rodok, vostavlennia jgo tiu.

Vikanuli UZD pecheni u B-regime z elastografie vo regime realnogo chasu na aparate Hitachi Hi Vision Avius. Nawnist i stopiń fibrozou oznqinili, vikoristovuvach klasifikatsiinu (diskriminantnu) lichilinu shkalu Bonacini, shki METAVIR i Ishak. Takoz vikonali korrelatsiin analiz rezultativ rizhnik metodik oznchennia stavdi fibrozou pechen i xorhiv na CD 2 tiu ta NAXHP.

Ruzultati. U bilišnosti xorhiv na CD 2 tiu za naavnost NAXHP diagnostuvali fibrozou slabogo stopiń (F0–F2). Oczhnia intesivnosti fibrozou pechen za danimi sofoelastografi i inimi neihivaznymi metodimy (shkali Bonacini, Ishak ta METAVIR) dalo možlivist otprimati zastavne rezultati. Ruzultati oznchennia stavdi fibrozou pechen vi obstekhenih xorhiv za danimi sofoelastografi naiokhlyše kerelou iz rezultatimy klasifikatsiin (diskriminantn) lichilinu shkalu Bonacini. Ruzultati shkal Ishak i METAVIR maю korrelatsiin zavizy serediniu stopiń z danimi sofoelastografi, a ičlii korrelatsiin zavizy vostavlil sami dli važnoghi stopiń fibrozou pecheni.

Visnovky. Ruzultati, co odenkali, zašaljai javljivost oznchennia kriiterij naavnosti avšioutsit NAXHP u xorhiv na CD 2 tiu, neobkhodimih diagnostich stadi NAXHP i vizhennia stavdi fibrozou pecheni. Poslednje U兹D diagnostiki ta shkvalivych biomarek, zašusosenia diagnostich shkal j znich informativniym i dočilnim dli oznchennia naavnost fibrozou pecheni v paientn iz NAXHP pörjëvno zia zašusovenamych cihh metodip poindnic, co takoz da zmëg zmenitju čostotu vikoristannya iňazivnych traumaticnih metodiv.
Diabetes mellitus (DM) is the most serious threat for the population all over the world. According to the World Health Organization, it is ranked third among the leading risk factors for premature death after arterial hypertension and smoking. International Diabetes Federation reveals that approximately 4 million people aged between 20 and 79 years in the world died in 2017 due to DM. The prevalence of diagnosed diabetes is 425 million people or 8.6 % of adult population (20–79 years) in the world. 212.4 million people or 50.0 % of all people aged 20–79 years with DM are unaware of their disease [1].

Non-alcoholic fatty liver disease (NAFLD) is pathogenetically associated with DM, its prevalence reaches 88 % in diabetic patients, while its prevalence is no more than 30 % in the general population [2–6].

At present, the pathogenesis of NAFLD is explained by the concept of “multiple impact”, which means the set of factors to be the reason of NAFLD development [7,8].

It is generally accepted that steatohepatitis has no tendency to progress, or it occurs very slowly. At the same time, the development of liver cirrhosis is relatively fast in patients with non-alcoholic steatohepatitis (NASH) [9–11]. Thus, it is critically important to determine the stage of the disease development and estimate the presence of liver fibrosis.

So far, there are many debatable questions regarding the use of non-invasive and minimally invasive methods for diagnosing various diseases, including NAFLD, using biomarkers. It is necessary to find the most informative non-invasive diagnostic methods and implement them into clinical practice for equivalent replacement of traumatic and potentially dangerous instrumental methods.

Materials and methods

The study involved 43 patients with type 2 DM and NAFLD, including 26 females (60.5 %) and 17 males (39.5 %). The average age of patients was 55.9 ± 1.3 years. All patients were examined and treated in the Si “V. Danilevsky Institute for Endocrine Pathology Problems of the NAMS of Ukraine”, Kharkiv.

The following inclusion criteria were applied: a written informed consent obtained from each patient to participate in the study, verified diagnosis of type 2 DM, verified diagnosis of NAFLD. Exclusion criteria were a diagnosis of type 1 DM, alcoholic liver disease, hepatitis C infection.

All patients underwent an extensive ultrasound examination of the liver, namely determination of its size below the costal arch, a duplex examination to assess the location of hepatic vessels, determine possible blood flow trouble and its type, etc. B-mode liver ultrasound with real-time elastography on Hitachi Hi Vision Avius apparatus was also performed.

The assessment of fibrosis presence and its stage was performed using the Bonacini discriminant score [23]. For evaluating the index of fibrosis according to this scoring system, the INR, AIAT/AsAT ratio and platelet count were determined. Based on the fibrosis index values, we assessed the intensity of fibrosis and correspondence between the stage of fibrosis and the results of histological evaluation by METAVIR and Ishak scores [17,18].

A correlation analysis between the results of various methods for assessing the stage of liver fibrosis in patients with type 2 DM in the presence of NAFLD was done.

The principles of bioethics were taken into account in the study: the general principles of the Council of Europe Convention on Human Rights and Biomedicine (04.04.1997), the GCP (1996), the ethical principles of medical research involving human subjects of the World Medical Association (04.04.1997), the GCP (1996), the ethical principles of medical research involving human subjects of the World Medical Association.
Table 1. Indexes of liver ultrasound examination in patients with type 2 DM and NAFLD, M ± m

<table>
<thead>
<tr>
<th>Indexes</th>
<th>Males, n = 19</th>
<th>Females, n = 28</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatomegaly, cm</td>
<td>2.34 ± 0.37</td>
<td>1.59 ± 0.34</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Diameter of total bile duct, mm</td>
<td>6.11 ± 0.02</td>
<td>6.22 ± 0.05</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Diameter of spleen vein, mm</td>
<td>6.79 ± 0.10</td>
<td>6.48 ± 0.13</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Diameter of portal vein, mm</td>
<td>11.61 ± 0.17</td>
<td>11.59 ± 0.20</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Velocity of blood flow in the portal vein, cm/sec</td>
<td>13.82 ± 0.19</td>
<td>13.87 ± 0.16</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Index of liver density according to sonoelastography Me [Q1;Q3]</td>
<td>2.00 [1.00; 2.25]</td>
<td>2.0 [1.0; 2.0]</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

Table 2. Comparative analysis of fibrosis stage by the results of elastography and other non-invasive methods, M ± m

<table>
<thead>
<tr>
<th>Staging of liver fibrosis, points</th>
<th>Males</th>
<th>Females</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Index of liver density according to sonoelastography</td>
<td>2.63 ± 0.05</td>
<td>2.49 ± 0.06</td>
<td>0.05</td>
</tr>
<tr>
<td>Bonacini discriminant score</td>
<td>2.29 ± 0.32</td>
<td>2.45 ± 0.25</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Fibrosis stage by Ishak score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>min</td>
<td>0.64 ± 0.34</td>
<td>0.63 ± 0.29</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>max</td>
<td>2.5 ± 0.39</td>
<td>2.53 ± 0.32</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Fibrosis stage by METAVIR score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>min</td>
<td>0.43 ± 0.23</td>
<td>0.38 ± 0.18</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>max</td>
<td>1.36 ± 0.25</td>
<td>1.33 ± 0.19</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

Table 3. Results of comparative analysis of liver fibrosis stage in patients with type 2 DM and NAFLD by various non-invasive methods, n (%)

<table>
<thead>
<tr>
<th>Method for determining liver fibrosis</th>
<th>Intensity of liver fibrosis</th>
<th>Males, n = 17</th>
<th>Females, n = 26</th>
<th>χ²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elastography</td>
<td>Mild fibrosis</td>
<td>12 (70.6 %)</td>
<td>15 (57.7 %)</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>Moderate fibrosis</td>
<td>5 (29.4 %)</td>
<td>11 (42.3 %)</td>
<td></td>
</tr>
<tr>
<td>METAVIR score</td>
<td>Mild fibrosis</td>
<td>11 (64.7 %)</td>
<td>17 (65.4 %)</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td>Moderate fibrosis</td>
<td>6 (35.3 %)</td>
<td>9 (34.6 %)</td>
<td></td>
</tr>
<tr>
<td>Ishak score</td>
<td>Mild fibrosis</td>
<td>11 (64.7 %)</td>
<td>15 (57.7 %)</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td>Moderate fibrosis</td>
<td>6 (35.3 %)</td>
<td>11 (42.3 %)</td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Correlation analysis of the liver fibrosis stages detected in patients with type 2 DM and NAFLD based on sonoelastography and other non-invasive methods (rxy ± mrxy)

<table>
<thead>
<tr>
<th>Correlation pairs</th>
<th>Results of elastography</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All patients</td>
</tr>
<tr>
<td>Density index</td>
<td>0.77 ± 0.17, P &lt; 0.001</td>
</tr>
<tr>
<td>Bonacini</td>
<td>0.68 ± 0.13, P &lt; 0.001</td>
</tr>
<tr>
<td>Ishak max</td>
<td>0.36 ± 0.17, P &lt; 0.05</td>
</tr>
<tr>
<td>Ishak min</td>
<td>0.50 ± 0.16, P &lt; 0.01</td>
</tr>
<tr>
<td>METAVIR min</td>
<td>0.37 ± 0.16, P &lt; 0.05</td>
</tr>
<tr>
<td>METAVIR max</td>
<td>0.47 ± 0.15, P &lt; 0.01</td>
</tr>
</tbody>
</table>


The study results were processed using Microsoft Excel 2010 and StatPlus Pro 5 (6.7.1.0). The adequacy of the parameters to normal distribution was tested using the Shapiro–Wilk and Kolmogorov-Smirnov tests. Descriptive statistics parameters for continuous variables were presented as the arithmetic mean and standard deviation. Results, which did not follow normal distribution, were expressed as median and interquartile range. The significance of differences was evaluated using Student’s t-criterion for independent samples in a normal distribution or the Mann-Whitney U-test for independent samples in a distribution different from normal. The relationship between two variables was measured by using Spearman correlation coefficient. A P value <0.05 was considered statistically significant.

Results

An assessment of the liver morphological state was performed and the stages of fibrosis were determined in patients with type 2 DM and NAFLD (Table 1). The degree of liver enlargement, its structure, the size of the total bile duct, splenic and portal veins, the velocity of blood flow in the portal vein, as well as the liver density index according to the sonoelastography, were evaluated.

This data demonstrates a liver enlargement of 1 cm to 6 cm, mainly of the right lobe. On average, it was 2.34 ± 0.37 cm in males and 1.59 ± 0.34 cm in females. There was no significant difference between men and women according to this indicator.

In the examined patients, the liver predominantly had a hyperechoic, coarse-grained structure that was diagnosed in 13 males (68.4 %) and 19 females (67.9 %). A hypoechoic liver structure was detected in 6 men (31.6 %) and 9 women (32.1 %). Such data testifies the development of fibrotic changes in the liver of patients with type 2 DM, which are typical for NAFLD.

The diameter of the total bile duct in men was on average 6.11 ± 0.02 mm, it was higher than that in women (6.22 ± 0.05). The mean diameters of the splenic vein in men and women were 6.79 ± 0.10 and 6.48 ± 0.13 mm, respectively. The portal vein diameter in the examined patients was 11.61 ± 0.17 for men and 11.59 ± 0.20 for women on average. The portal vein blood flow velocity in the examined patients was 13.82 ± 0.19 cm/sec in men and 13.87 ± 0.16 cm/sec in women on average. The liver density index according to the sonoelastography data in the examined men was 2.0 [1.00; 2.25] points, and in women this figure was 2.0 [1.0; 2.0] points as well.

A comparative analysis of the elastography results and detection of the liver fibrosis stage based on the Bonacini, Ishak and METAVIR scores was performed (Table 2).

The data given demonstrate that the liver density index by sonoelastography and results of other methods did not differ significantly in men and women. Thus, the stages of fibrosis according to the Bonacini discriminant score were 2.29 ± 0.32 points in males and 2.45 ± 0.25 in women. The detection of fibrosis stage according to the Ishak scores in the examined men and women was 0.64 ± 0.34 and 0.63 ± 0.29 points (minimum values) and 2.50 ± 0.39 and 2.53 ± 0.32 points (maximum values), respectively. The severity of fibrosis manifestations by the METAVIR score was estimated, on average, as 0.43 ± 0.23 points in males and 0.38 ± 0.18 points in women (minimum values) and 1.36 ± 0.25 points in males and 1.33 ± 0.19 points in women (maximum values).

The number of patients with mild and moderate liver fibrosis diagnosed according to various non-invasive techniques is presented in Table 3.

We can see that in the predominant number of examined patients with type 2 DM and NAFLD, a mild degree
Discussion

The real prevalence of NASH and fibrosis remains uncertain, as the diagnosis requires a liver morphological examination. The gold diagnostic standard for NAFLD is liver biopsy, which allows assessing the stage of fibrosis and determining the presence of steatosis or NASH [12, 13].

Methods of histological analysis include evaluation the fibrosis stage by Brunt [14], NASH CRN [15], HAI-Knodel methods (Bonacini, Ishak and METAVIR scores) allowed us to obtain similar results. The results of liver fibrosis stage assessment in patients with type 2 DM according to sonoelastography were most strongly correlated with the results of the Bonacini classification (discriminant) score. The results of liver fibrosis stage assessment in patients with type 2 DM and NAFLD, mild fibrosis (F0–F2) was diagnosed. The combination of ultrasound diagnosis, serum biomarkers and use of diagnostic scales is more informative and appropriate for assessing the liver fibrosis presence in patients with NAFLD, compared with separate use of these methods allowing reducing the frequency of invasive traumatic methods using. In the vast majority of examined patients with type 2 DM and NAFLD, mild fibrosis was diagnosed. The estimation of the liver fibrosis stage according to sonoelastography and other non-invasive methods allowed obtaining comparable results.

In order to estimate the stage of fibrosis in patients with NAFLD, it is advisable to use sonoelastography and the Bonacini discriminant score, which have similar significance complementing each other and contribute greatly to detecting the very early manifestations of liver fibrosis in patients with type 2 DM and NAFLD.

Conclusions

1. Generally, in the examined patients with type 2 DM and NAFLD, mild fibrosis (F0–F2) was diagnosed. The estimation of the liver fibrosis stage according to sonoelastography and other non-invasive methods (Bonacini, Ishak and METAVIR scores) allowed to get comparable results.

2. The results of liver fibrosis stage assessment in patients with type 2 DM in the presence of NAFLD according to sonoelastography were most strongly correlated with the results of the Bonacini classification (discriminant) score. The results of the Ishak and METAVIR scores had a moderate correlation with the sonoelastography data. Strong correlations were detected in advanced stages of hepatic fibrosis.

Prospects for further research. It is planned to continue the study of various fibrosis markers and results of liver puncture biopsy.

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References


