MODERN METHODS OF DIAGNOSIS AND SCREENING OF NON-ALCOHOLIC FATTY LIVER DISEASE AND ITS STAGES (REVIEW)

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Abstract

The review features the problem of diagnosing one of the most common pathologies of the contemporary world – non-alcoholic fatty liver disease (NAFLD). Data from experimental and clinical studies on the importance of various instrumental and biochemical methods of non-invasive diagnosis of non-alcoholic steatohepatitis (NASH) and liver fibrosis (LF) are presented. New non-invasive diagnostic methods of NASH and LF are discussed.

Keywords: *nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, liver fibrosis.*

In the structure of the overall morbidity in the economically developed countries of the world, nonalcoholic fatty liver disease (NAFLD) occupies one of the leading positions, pushing the hepatitis of viral and alcoholic origin [1, 2]. Despite the long period of study of NAFLD, timely diagnosis of its stages remains imperfect. This is mainly due to the diagnosis of NAFLD based on the history data, clinical and laboratory studies, and their interpretation, and much less often on the basis of the study of specific biomarkers of this pathology. The most common and adequate method of diagnosing nonalcoholic steatohepatitis (NASH) and liver fibrosis (LF) in patients with NAFLD is puncture liver biopsy [3, 4]. However, in most cases, a number of limitations for puncture liver biopsy should be considered. Such limitations include invasiveness of the procedure, its cost, diagnostic errors associated with the location of the sample, the presence of contraindications associated with the procedure, the risk of complications and mortality, etc. [5]. This number of limitations of liver biopsy does not allow the use of this procedure for the current screening of NASH and LF in patients with NAFLD.

Morphological examination of the liver allows to directly assess not only the stage of fibrosis but also a number of other indicators of liver damage: the presence of steatosis, inflammation, accumulation of copper, iron, and other histological changes. In complex diagnostic cases, histological findings are crucial for diagnosis verification. One of the major disadvantages of liver biopsy, which limits its use, is the presence of contraindications and the risk of complications. Absolute contraindications for liver biopsy are the presence of vascular malformations, obstruction of the extrahepatic bile ducts (biliary obstruction), uncompensated coagulation disorders, cystic changes in the liver [6]. Relative contraindications for liver biopsy are the presence of severe ascites, obesity, hemophilia, liver amyloidosis, right pleurisy or subphrenic abscess on the right, bacterial cholangitis [7].

Frequent complications of liver biopsy are pain in the right upper quadrant of the abdomen, intrahepatic or subcapsular hematoma, hypotension associated with the vasovagal reaction, intraperitoneal bleeding, biliary peritonitis. According to the data of the National Health Service of the United Kingdom, collected from 1998 to 2005, among 61,187 patients who underwent liver biopsy, the overall mortality rate was 2 cases per 1,000 biopsies (95%, confidence interval 1.8 – 2.5) [8].

Diagnostic errors in assessing the histological activity and degree of fibrosis due to uneven distribution of fibrous tissue are also possible. Thus, in the study of MD. Federica Vernuccio (2019), the diagnostic accuracy of liver biopsy performed among 389 patients was 89.4%, and the incidence of false-negative results was 6.5% [9].

In modern medical practice, ultrasound of the liver is most widely used in the diagnosis of NAFLD. Ultrasonographic signs of hepatic steatosis are an increase in its echogenicity compared

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with the parenchyma of the cortical layer of the kidneys, a bright pattern with vascular erosion, which is determined by deep attenuation of the wave, and focal hepatosteatosis [10]. The undoubted advantages of liver ultrasound are its safety and relatively low cost, which makes it possible to conduct repeated studies. However, with hepatic steatosis < 20% and BMI > 40 kg/m2, the sensitivity and accuracy of liver ultrasound to verify the diagnosis of NAFLD is limited [11]. Despite the fact that the quality of ultrasound diagnosis depends on the experience and qualifications of the specialist, ultrasound can reliably diagnose moderate and severe steatosis and provides additional information about the state of the hepatobiliary system [12].

Contrast-enhanced computed tomography (CT) of the liver is of high diagnostic value in the diagnosis of NAFLD due to its availability, ease of use, and accuracy in imaging hepatic steatosis [13]. Contrast-enhanced liver CT is a more complex method in terms of quantifying liver fat deposition due to the imposed parenchymal enhancement of the liver CT signal [14]. However, CT of the liver with vein contrast is a diagnostically valuable method of diagnosing moderate and severe steatosis in patients with NAFLD [187]. However, CT-scans cannot detect the initial LF. Also, the potential danger of ionizing radiation makes liver CT unsuitable for long-term follow-up of patients with NAFLD [15].

Another visualization method in NAFLD is magnetic resonance imaging (MRI) of the liver. Standard MRI of the liver, including chemical shift imaging with input and reverse phases, is diagnostically justified for the diagnosis of hepatic steatosis as a whole, but this method does not provide data on objective quantification of liver fat [16]. Until recently, MR spectroscopy was the reference standard for non-invasive imaging and quantification of liver fat. However, this method takes a lot of time and, as in the case of liver biopsy, is prone to errors in data interpretation [17].

Elastography is a very effective modern method of radiological diagnosis of NAFLD and its stages. Elastography has the ability to demonstrate increased stiffness of the liver parenchyma as a result of inflammation or fibrotic changes in the liver [18]. One of elastography types is transient elastography (TE). TE is a non-invasive technique recommended as an alternative method of morphological examination of the liver, which allows quick assessment of the presence of LF, including in the dynamics [19]. Also, TE is a method of imaging that allows non-invasive assessment of the stage of LF in patients with NAFLD, especially in patients with severe fibrosis and liver cirrhosis. However, the main disadvantage of TE is the unreliability of the results in patients with high BMI and/or significant thickening of the chest folds.

One of the leading methods of quantitative elastography of the liver is transitional elastography under the trademark "FibroScan". The method is based on the determination of liver fibrosis with the propagation of elastic waves from 20-30 ultrasonic pulses, followed by calculation of the average value of the deformation pressure in kilopascals (kPa) [20]. Maximum diagnostic accuracy of elastography was achieved in patients with LF stage F3 and F4 based on the results of semi-quantitative assessment of fibrosis (histological scale Metavir). Informativeness of the method by stages of liver fibrosis: F0-F1 – 88-90%, F2- F3 – 90-94, F4 – 94-98% [21].

However, the procedure is not recommended for patients with pacemakers and pregnant women due to the high acoustic power of the pulse. Also, this method has a high cost and does not give the exact location of the area of interest, as it is performed "blindly" and has a depth limit of 5 cm with a fixed size of the control volume of 4 cm [22]. A significant limitation of the method is the reduction in the significance of the results in overweight patients, and given that most patients with NAFLD have concomitant, this circumstance is a significant disadvantage [23].

Different scores and biomarkers are also used for the non-invasive diagnosis of NAFLD. One such biomarker for calculating hepatic steatosis is the fatty liver index. The liver obesity index has been reported to be a predictor of insulin resistance and is closely related to NAFLD [24].

Another scale for verifying hepatic steatosis is the NAFLD liver fat score. This scale is a reliable prognostic scale for predicting fat dep osition in the liver (AUROC 0.775-0.786) [25].

A hepatic steatosis index is also available to assess hepatic steatosis in NAFLD. The hepatic steatosis index has been reported to be an indirect marker of hepatic steatosis and metabolic syndrome in patients with NAFLD. In a study conducted by Jun Hyung Kim (2020), sensitivity of hepatic steatosis index was 90%; specificity – 74%; plausibility ratio – 3.46; positive prognostic value – 0.64; and negative prognostic value of 0.93 [26]. The result of calculating the index of hepatic steatosis in the range of 30 - 36 may indicate the presence of NAFLD in the stage of steatosis. Accordingly, at values < 30 or> 36 – NAFLD is not diagnosed [27].

According to the literature, there are also indirect biochemical markers of NAFLD – molecules that are released into the blood due to a pathological process occurring in the liver and are also able to reflect the presence of inflammation and its activity. They are represented by aminotransferases ALT and AST; molecules synthesized in hepatocytes by the liver, for example, alkaline phosphatase (AP), gamma-glutamyl transpeptidase (GGT), apolipoprotein A1, alpha-2-macroglobulin (A2M), ferritin, haptoglobin, coagulation factors.

The standard panel of a comprehensive study of the functional state of the liver includes measurements of AST, ALT, AP, total bilirubin and serum albumin. ALT and AST are liver enzymes involved in the transfer of the amino groups of aspartate and alanine to ketoglutaric acid and are markers of hepatocellular damage [28]. AST activity is most pronounced in the liver, heart muscle, kidney, and brain tissue, while ALT activity is predominant in liver tissue, making elevated ALT levels a more specific marker of hepatocyte damage. Numerous studies have shown that elevated ALT levels are associated with increased mortality in patients with liver disease, including NAFLD [29]. Also, a predictor of the severity of the liver disease is the ratio of AST and ALT, the so-called de Ritis coefficient, the value of which more than one (1), may indicate the presence of severe fibrotic changes in the liver in patients with NAFLD [30].

AP is a part of the family of enzymes of zinc metalloproteinases, which catalyze hydrolysis of esters of phosphoric acid under alkaline pH. This enzyme is found in hepatocytes on the tubular membrane, as well as in bone, placenta, intestine and kidneys. An isolated increase in AP levels can be observed after eating fatty foods, bile duct obstruction, pregnancy, and liver damage [31]. In the case of increased AP levels, to confirm the damage to liver tissue, it is necessary to further determine the level of tubular liver enzyme – GGT. Increased AP levels in combination with elevated GGT levels reliably indicate the process of hepatocyte damage, including NAFLD [32].

Total bilirubin is synthesized as a result of the physiological breakdown of erythrocytes and circulates in the unconjugated form. Unconjugated bilirubin, according to the Van den Berg reaction, is defined as indirect, accounting for about 70% of total serum bilirubin. There is scientific evidence that an increase in total bilirubin is associated with a risk of cardiovascular diseases (CVD), diabetes mellitus type 2 and metabolic syndrome [33]. Also, there are data associating with increase of circulating bilirubin with the development of NAFLD and the risk of NASH progression [34].

Apolipoprotein A1 is a 243-amino acid polypeptide that is mainly present in plasma as a component of HDL and is controversial as a marker of NAFLD and its stages. Elevated serum apolipoprotein A1 has been reported to be significantly associated with the development of NAFLD, regardless of the presence of metabolic syndrome [35]. However, a study by Reza Fadaei (2018) conducted among 50 patients with histologically confirmed NAFLD showed that circulating apolipoprotein A1 levels were lower in the NAFLD group compared to the control group [36].

Haptoglobin, first described by Polonowski and Jail, is a tetra-chain glycoprotein that normally circulates in blood plasma in the amount of 0.3 – 3 g/l. Haptoglobin is considered a marker of acute inflammation, which is synthesized in the liver and immune cells, including neutrophils and monocytes. Accumulated data on the function of this protein has established its close relationship with non-communicable diseases, which are based on the development of chronic systemic inflammation (obesity, CVD, arterial hypertension) [37]. It is well known that determination of haptoglobin is included in the panel of biochemical markers for determination of AF "FibroTest" and "Actitest" in patients with NAFLD. Chwist A. et al. (2014) reported that the level of haptoglobin

was significantly higher in the group of patients with NAFLD in the LF stage F2-F3 compared with the group of patients with NAFLD in the LF stage F0-F1and the control group [38].

Thus, there are a significant number of invasive and non-invasive methods for diagnosing NAFLD. However, the application of these methods presents certain difficulties due to their complexity, significant risk of complications, high probability of subjectivity and erroneous judgments in the interpretation of results, low patient compliance, inability to use in dynamics and high cost of research.

It should also be noted that as of today, the number of studies on diagnostic tactics in patients with NAFLD is insignificant, and the question of non-invasive diagnosis of NAFLD remains open.

Thus, the future of diagnostic hepatology is the use of non-invasive methods of diagnosing NAFLD using specific serum biomarkers with the possibility of early non-invasive diagnosis of NAFLD and differentiation of steatosis, NASH and LF.

Declarations

Statement of Ethics

The author has no ethical conflicts to disclosure.

Consent for publication

The author gives her consent to publication.

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