Clinical research

Non-invasive diagnosis of steatosis, inflammatory changes and liver fibrosis in patients with non-alcoholic fatty liver diseases. Pilot study

Irena Ciećko-Michalska[1], Małgorzata Szczepanek[2], Iga Wierzbicka-Tutka[1], Janina Zahradnik-Bilska[3], Tomasz Mach[1]

[1]Department of Gastroenterology, Hepatology and Infectious Diseases, Jagiellonian University Medical College, Krakow, Poland
[2]Department of Medical Didactics, Jagiellonian University Medical College, Krakow, Poland
[3]University Hospital, Krakow, Poland

Corresponding author:
Irena Ciećko-Michalska MD
Department of Gastroenterology, Hepatology and Infectious Diseases
Jagiellonian University Medical College
5 Śniadeckich St
31-531 Krakow, Poland
Phone: +48 12 424 73 82
E-mail: michalska@su.krakow.pl

Submitted: 7 June 2018
Accepted: 11 December 2018

Arch Med Sci Atheroscler Dis 2018; 3: e179–e183
DOI: https://doi.org/10.5114/amsad.2018.81184

Abstract

Introduction: Nonalcoholic fatty liver disease (NAFLD) is one of the most common causes of abnormal liver enzymes in adult patients consulted by hepatologists. Due to the high prevalence of this disease, most often associated with obesity, it is necessary to assess the risk of NAFLD, monitoring the progression of the disease and the effectiveness of treatment.
Material and methods: We evaluated the intensity of steatosis, inflammatory activity and fibrosis in 36 patients with NAFLD (fatty liver in abdominal ultrasound examination), using non-invasive tests: SteatoTest, ActiTest and FibroTest. We compared the prevalence of metabolic disorders and hypertension between women and men.
Results: There were no significant differences in analysed parameters of metabolic disorders between women and men. In both studied groups, the intensity of steatosis and inflammatory changes was similar. However, in the male group, the intensity of liver fibrosis was higher.
Conclusions: The tests helped to detect advanced liver fibrosis in patients who were diagnosed with liver steatosis in ultrasound examination. Non-invasive diagnostics of liver injury may be useful in screening to select groups of patients requiring liver biopsy, as well as in monitoring the course of the disease and assessment of the treatment effectiveness. Early detection of liver disease may improve the prognosis of these patients.

Key words: non-alcoholic fatty liver disease, SteatoTest, ActiTest, FibroTest, non-invasive diagnosis, liver fibrosis.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease all over the world. It covers the spectrum of disorders such as non-alcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH) without or with fibrosis, liver cirrhosis (LC), and hepatocellular carcinoma (HCC) [1]. The NAFL is the result of an imbalance between the formation of triglycerides (TG) and their utilization [2]. It is associated with excessive accumulation of TG in hepatocytes due to excessive inflow of free fatty acids and *de novo* lipogenesis and impaired TG ex-

port from the liver in the form of very low density lipoprotein (VLDL), which increases the pool of lipids accumulated in the liver [2, 3]. Enlarged adipocytes in adipose tissue decrease the ability to store fat and are resistant to the anti-lipolytic effect of insulin [4]. Insulin resistance is considered to be the key pathophysiological factor in NAFLD. In addition, increased lipid peroxidation, reactive oxygen species (ROS) and oxidative stress stimulate the pro-inflammatory response in the liver [5]. This leads to the development of NASH with LC and HCC. It has been demonstrated that HCC develops five times more often in liver without cirrhosis in patients with NAFLD than in other aetiologies [6]. The risk factors for NAFLD are metabolic syndrome, male sex, age over 50 years, type 2 diabetes (T2DM), hypertension and increased alanine aminotransferase (ALT) activity [1].

The NAFLD occurs in 95% of obese people [7] and in 7% of people with normal body weight [8]. It is considered to be a liver manifestation of the metabolic syndrome, which is a combination of factors that increase the risk of atherosclerosis, type 2 diabetes, and cardiovascular complications [9, 10].

In the POLSenior population study, NAFL was diagnosed in 37.2% of the population (> 65 years old), and 14.8% of people had advanced liver fibrosis, which increased with age [11].

Liver biopsy is the only test that enables unquestionable differentiation between NAFL and NASH [12]. However, it is recommended in patients with NAFLD with an increased risk of NASH and advanced fibrosis, and in patients with suspicion of NAFLD, in whom without biopsy another aetiology of the liver steatosis or coexistence of other chronic liver diseases cannot be ruled out [1, 13].

Taking into consideration the aging of societies and the increasing prevalence of obesity and diabetes, a further increase in the prevalence of NAFLD over the next decades should be assumed. Therefore, basic imaging examinations such as ultrasound (USG), which is, according to current recommendations, the basic imaging study in the diagnosis of NAFLD and non-invasive diagnostic tests for assessing the severity of fatty liver and fibrosis in the liver are used in screening and population studies [1]. Non-invasive diagnostic tests may be useful in the early detection of liver damage, prognosis of the course of the disease and assessment of the effectiveness of treatment.

FibroMax (BioPredictive, Paris, France) is one such non-invasive test. It is considered a universal biomarker for chronic liver disease, and includes a combination of FibroTest for quantitative fibrosis assessment, SteatoTest for quantitative steatosis and ActiTest for the quantitative evaluation of inflammatory activity. These tests may be useful in assessing the risk of fibrosis and cirrhosis in people with chronic liver disease. FibroMax was validated in a FLIP (Fatty liver: Inhibition of Progression) prospective cohort study in patients with histologically confirmed NAFLD [14]. The results of the studies showed that FibroTest, ActiTest and SteatoTest have very high compliance with histological results in NAFLD; hence they can be an alternative to liver biopsy [14, 15].

SteatoTest, ActiTest and FibroTest have been patented as in vitro diagnostic tests for the diagnosis of fibrosis stages in relation to the METAVIR scale [14].

The aim of this study was to assess the intensity of steatosis, inflammatory activity and fibrosis using FibroMax in patients with hepatic steatosis in USG and to compare the results between the group of women and men.

Material and methods

We examined 36 patients who came for consultation due to fatty liver in the USG with normal or elevated liver enzymes activity: 19 men (21–51 years old, body mass index (BMI): 20.8–34.1 kg/m^2) and 17 women (27–64 years old, BMI: 20.3–33.9 kg/m^2) – Table I. All participants expressed their written consent to participate in the study. A questionnaire regarding alcohol consumption, coexistence of dyslipidaemia, diabetes mellitus, hypertension and medication was carried out in all participants. People with a history of alcohol consumption > 20 g/day, viral and autoimmune liver disease were excluded from our study. The

Table I. Demographic data and concomitant diseases in the group of women and men

Parameter	Total	Women	Men
Group size	36	17	19
Mean age (min.–max.) [years]	44.1 (21–64)	49.8 (27–64)	39 (21–51)
BMI (min.–max.) [kg/m^2]	27.5 (20.3–38.1)	25.5 (19.8–38.1)	29.7 (20.8–34.1)
Duration of disease (min.–max.) [years]	6.3 (1–31)	8 (1–31)	4.8 (1–15)
Fasting hyperglycaemia/T2DM	9	4	5
Hypertension	8	4	4

Table II. Results of laboratory tests in the group of women and men

Parameter (laboratory standards)	Mean total (min.–max.)	Mean female (min.–max.)	Mean male (min.–max.)	*P*-value F/M
Bilirubin (0.0–21.0 mmol/l)	15.2 (6–41)	12.8 (6–23)	17.4 (7–41)	0.1
GGT (F: 5–36; M: 8–61 U/l)	119.1 (23–477)	95 (23–457)	140.6 (24–477)	0.057
ALT (F: 5–33; M: 5–41 U/l)	60 (15–220)	62.6 (15–220)	57.6 (14–140)	0.72
AST (F: 5–32; M: 5–40 IU/l)	50.7 (19–171)	53.5 (19–171)	48.2 (20–161)	0.69
Fasting glucose (3.3–5.6 mmol/l)	5.8 (3.7–10.8)	6.2 (4.4–10.8)	5.4 (3.7–7.8)	0.23
Total cholesterol (3.2–5.2 mmol/l)	5.3 (3.15–8.03)	5.0 (3.1–7.8)	5.5 (4.1–8.0)	0.3
Triglycerides (< 2.26 mmol/l)	1.7 (0.57–3.11)	1.6 (0.6–3.1)	1.9 (0.7–2.8)	0.29
FibroTest	0.31 (0.03–0.78)	0.28 (0.03–0.78)	0.33 (0.05–0.78)	0.44
ActiTest	0.355 (0.04–0.94)	0.33 (0.04–0.93)	0.37 (0.09–0.94)	0.65
SteatoTest	0.55 (0.11–0.88)	0.54 (0.11–0.88)	0.56 (0.2–0.83)	0.73

height and weight of all participants were evaluated and blood samples were collected to determine activity of liver enzymes and FibroMax, including: FibroTest, ActiTest and SteatoTest, producer: BioPredictive, Paris, France [16].

The results of these tests are calculated as algorithms of clinical and biochemical parameters in the range of 0–1. FibroTest is calculated based on the concentration of α2-macroglobulin, haptoglobin, apolipoprotein A1, total bilirubin and GGT, adjusted for age and sex. ActiTest additionally includes ALT, and ST: additionally total cholesterol, fasting glucose, triglycerides, height and weight [17].

Statistical analysis

The results of the study were statistically analysed using free software for statistical computing and graphics – the R program.

Results

In the studied group M vs. W hypercholesterolaemia was found in 21% vs. 23%, hypertriglyceridaemia in 32% vs. 28%, fasting hyperglycaemia 47% vs. 41%, hypertension in 21% vs. 23% of patients. In SteatoTest: no or minimal steatosis was observed in 31% of men vs. 35% of women, significant or severe in 69% of men vs. 65% of women. In ActiTest: men vs. women, no or mild inflammatory activity (A0–A1) was found in 57% vs. 59%, moderate and severe (A2–A3) in 43% vs. 41% of subjects. In FibroTest: men vs. women no or moderate fibrosis (F0–F2) was found in 79% vs. 88% advanced (F3) in 21% vs. 12% of patients (Table II).

The results of our study did not show statistically significant differences in the analysed metabolic disorders between groups of women and men. In both groups, the intensity of steatosis and inflammatory activity was similar. However, in the male group, a greater degree of fibrosis was observed (Figures 1–3).

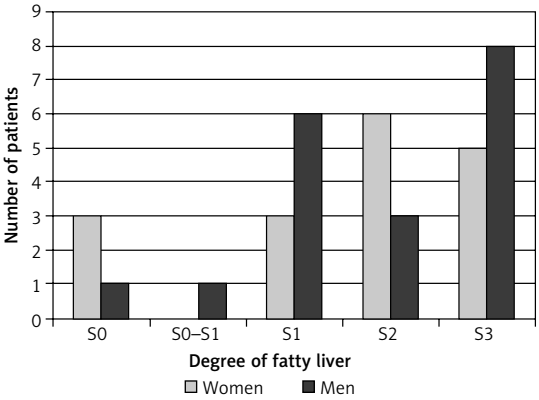

Figure 1. Degree of fatty liver in the group of women and men. Interpretation of the SteatoTest: S0 – no steatosis, S1 – minimal (1–5%), significant S2 (6–32%), heavy S3 (> 32%)

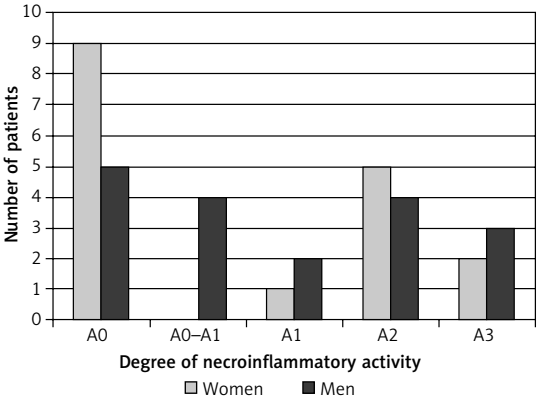

Figure 2. Degree of necroinflammatory activity in the group of women and men. Interpretation of the ActiTest: assignment of results to the degree of fibrosis in histological classification METAVIR 1.00 (A3), 0.61–0.62 (A2–A3), 0.53–0.60 (A1–A2), 0.53–0.60 (A2), 0.37–0.52 (A1–A2), 0.30–0.36 (A1), 0.18–0.29 (A0–A1), 0.00–0.17 (A0)

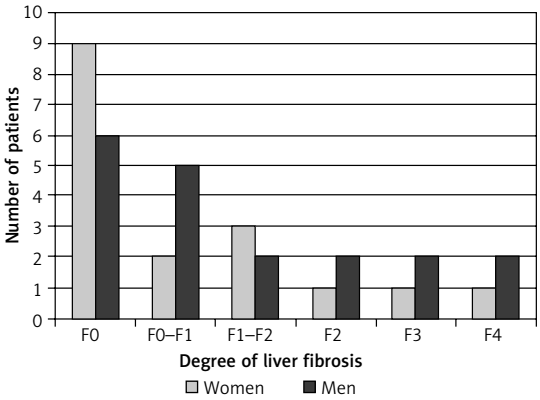

Figure 3. Degree of liver fibrosis in the group of women and men. Interpretation of the FibroTest: assignment of results to the degree of fibrosis in histological classification METAVIR: 0.75–1.00 (F4), 0.73–0.74 (F3–F4), 0.59–0.72 (F3), 0.49–0.58 (F2), 0.32–0.48 (F1–F2), 0.28–0.31 (F1), 0.22–0.27 (F0–F1), 0.22–0.27 (F0–F1), 0.00–0.21 (F0)

Discussion

In our study, all examined patients were diagnosed with fatty liver in USG, and about 30% with metabolic syndrome. Elevated ALT activity was found in 71% of women and 69% of men, abnormal GGT in 65% of women and 74% of men. According to the currently valid criteria, it indicates that the majority of patients were NASH patients and not simple NAFL patients. Normal or periodically normal activity of liver enzymes and the incidence of their elevation probably do not reflect the true prevalence of NAFLD [8]. In the population of the United States, an increase of transaminases was noted in 7.9% of subjects, more often in men than in women (9.35 vs. 6.6%); however, after exclusion of excessive alcohol consumption, hepatitis B and C and haemochromatosis, the cause of abnormalities was not identified in up to 69% of those examined. However the increase of transaminases was associted in both women and men groups withwith higher BMI, higher triglycerides, fasting glucose, type 2 diabetes and hypertension, which may indicate NAFLD [18], whereas elevated GGT activity is considered an NAFLD marker in patients with metabolic syndrome [19].

In our study BMI $\geq$ 25 kg/m^2 was observed in 82% of women and 89% of men. However, NAFLD was also observed in patients with normal body weight and it was independently associated with younger age, female sex, less likelihood of insulin resistance and hypercholesterolaemia [8]. Therefore, prophylactic ultrasound should be performed in both normal body weight and overweight or obese people with the aim of early detection of NAFL. The results of the meta-analysis of Hernaez *et al.* showed that ultrasound examination is an accurate and sensitive tool for the detection of moderate and severe liver steatosis with sensitivity and specificity of 84.8% and 93.6%, respectively [20]. In the NHANTES III population study conducted in the USA on a group of 20 050 participants, on the basis of clinical presentation, laboratory tests and ultrasound examination, NAFLD was diagnosed in 2492 people (18.77%), of whom 11.78% fulfilled NASH criteria [8]. Patients who have been diagnosed with hepatic steatosis without elevated liver enzymes should be evaluated for the presence of metabolic syndrome risk factors and other factors that may lead to steatosis, first of all alcoholic steatosis, whereas subjects with fatty liver in imaging examination and abnormal liver enzymes should be evaluated for NAFLD [21]. Non-invasive diagnostic tests can be used as first-line tests to rule out severe liver disease [1].

In our study, no or minimal liver steatosis was found in 1/3 of patients, and in 2/3 advanced or severe liver steatosis in women and men. Increased inflammatory activity was found in more than 40% of subjects, whereas advanced fibrosis was found almost twice as often in men (21%) than in women (12%).

A meta-analysis of 30 studies in which 6378 patients with FT and liver biopsies (3501 HCV, 1457 HBV, 267 NAFLD, 4229 ALD, 724 mixed aetiology) showed that the diagnostic value of FT, by analogy to the liver biopsy, was similar in the four most common aetiologies, and as in the case of a biopsy, it was higher in advanced fibrosis F2–F4 than F0–F1 [22]. Therefore, according to Poynard *et al.*, a liver biopsy recommended for millions of people at risk of fibrosis due to insufficient benefit/risk ratio (risk of death 0.3%) should be a second choice procedure for diagnostically difficult liver diseases [22].

The diagnostic usefulness of FibroTest, ActiTest and SteatoTest tests in people with severe obesity was also confirmed, which may also reduce the need for biopsy in this group of patients [14, 23].

The results of our research indicating a high percentage of patients with hepatic fibrosis referred to a hepatological outpatient clinic with fatty liver in USG and normal or abnormal liver enzymes confirm the importance of non-invasive diagnostics in screening tests, as it is impossible to perform liver biopsies in all such patients. Early detection of liver disease and introduced dietetic treatment, lifestyle modification, increased physical activity and weight loss, and intensification of treatment of metabolic syndrome, may significantly improve the prognosis of these people not only in preventing fibrosis, cirrhosis and HCC, but also in preventing cardiovascular complications.

In conclusion, the results of our study confirm that non-invasive diagnostics of liver injury in NAFLD may be useful as a screening method in people who have had fatty liver disease identified in an ultrasound examination. It can also be help-

ful in monitoring the course of the disease and assessing the effectiveness of treatment.

Acknowledgments

This paper was supported by a statutory grant from UJ CM no. K/ZDS/007184.

Conflict of interest

The authors declare no conflict of interest.

References

1. European Association for the Study of the Liver (EASL); 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. Obes Facts 2016; 9: 65-90.
2. Gan L, Xiang W, Xie B, Yu L. Molecular mechanisms of fatty liver in obesity. Front Med 2015; 9: 275-87.
3. Engin A Non-alcoholic fatty liver disease. Adv Exp Med Biol 2017; 960: 443-67.
4. Engin AB. What is lipotoxicity? Adv Exp Med Biol 2017; 960: 197-220.
5. Chen Z, Yu R, Xiong Y, Du F, Zhu S. A vicious circle between insulin resistance and inflammation in nonalcoholic fatty liver disease. Lipids Health Dis 2017; 16: 203.
6. Mittal S, El-Serag HB, Sada YH, et al. Hepatocellular carcinoma in the absence of cirrhosis in united states veterans is associated with nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol 2016; 14: 124-31.
7. Basaranoglu M, Kayacetin S, Yilmaz N, Kayacetin E, Tarcin O, Sonsuz A. World J Gastroenterol 2010; 16: 2223-6.
8. Younossi ZM, Stepanova M, Negro F, et al. Nonalcoholic fatty liver disease in lean individuals in the United States. Medicine (Baltimore) 2012; 91: 319-27.
9. Yu J, Marsh S, Hu J, Feng W, Wu C. The pathogenesis of nonalcoholic fatty liver disease: interplay between diet, gut microbiota, and genetic background. Gastroenterol Res Pract 2016; 2016: 2862173.
10. Ajmal MR, Yaccha M, Malik MA, et al. Prevalence of nonalcoholic fatty liver disease (NAFLD) in patients of cardiovascular diseases and its association with hs-CRP and TNF-alpha. Indian Heart J 2014; 66: 574-9.
11. Hartleb M, Barański K, Zejda J, Chudek J, Więcek A. Non-alcoholic fatty liver and advanced fibrosis in the elderly: results from a community-based Polish survey. Liver Int 2017; 37: 1706-14.
12. Ratziu V, Charlotte F, Heurtier A, et al. Sampling variability of liver biopsy in nonalcoholic fatty liver disease. Gastroenterology 2005; 128: 1898-906.
13. Hartleb M, Habior A, Cichoż-Lach H, et al.; członkowie Sekcji Hepatologicznej PTG-E. Znaczenie biopsji wątroby w praktyce klinicznej: rekomendacje Sekcji Hepatologicznej Polskiego Towarzystwa Gastroenterologii. Gastroenterol Prakt 2014; 6: 5-36.
14. Munteanu M, Tiniakos D, Anstee Q, et al.; FLIP Consortium and the FibroFrance Group. Diagnostic performance of FibroTest, SteatoTest and ActiTest in patients with NAFLD using the SAF score as histological reference. Aliment Pharmacol Ther 2016; 44: 877-89.
15. Lassailly G, Caiazzo R, Hollebecque A, et al. Validation of noninvasive biomarkers (FibroTest, SteatoTest, and Nash Test) for prediction of liver injury in patients with morbid obesity. Eur J Gastroenterol Hepatol 2011; 23: 499-506.
16. www.biopredictive.com/products/fibromax/
17. Pais R, Lupşor M, Poantă L, et al. Liver biopsy versus noninvasive methods – Fibroscan and Fibrotest in the diagnosis of non-alcoholic fatty liver disease: a review of the literature. Rom J Intern Med 2009; 47: 331-40.
18. Clark JM, Brancati FL, Diehl AM. The prevalence and etiology of elevated aminotransferase levels in the United States. Am J Gastroenterol 2003; 98: 960-7.
19. Banderas DZ, Escobedo J, Gonzalez E, Liceaga MG, Ramírez JC, Castro MG. gamma-Glutamyl transferase: a marker of nonalcoholic fatty liver disease in patients with the metabolic syndrome. Eur J Gastroenterol Hepatol 2012; 24: 805-10.
20. Hernaez R, Lazo M, Bonekamp S, et al. Diagnostic accuracy and reliability of ultrasonography for the detection of fatty liver: a meta-analysis. Hepatology 2011; 54: 1082-90.
21. Chalasani N, Younossi Z, Lavine JE, et al. The diagnosis and management of nonalcoholic fatty liver disease: practice guidance from the American Association for the Study of Liver Diseases. Hepatology 2018; 67: 328-57.
22. Poynard T, Morra R, Halfon P, et al. Meta-analyses of FibroTest diagnostic value in chronic liver disease. BMC Gastroenterol 2007; 7: 40.
23. Poynard T, Lassailly G, Diaz E, et al.; FLIP consortiumPerformance of biomarkers FibroTest, ActiTest, SteatoTest, and NashTest in patients with severe obesity: meta analysis of individual patient data. PLoS One 2012; 7: e30325.