DOI: https://doi.org/10.53350/pjmhs22162919

ORIGINAL ARTICLE

Comparison of NAFLD Fibrosis Score with Shear Wave Elastograpghy to Identify Liver Fibrosis in Patients with NAFLD

MUHAMMAD AZHAR WASEEM[1], SONIA SALEEM[2], MUHAMMAD YASIR YOUNIS[3], SAAD HASSAN KHAN[3], SADIA JABBAR[3], ASMAT ULLAH[4]

[1]*Senior Registrar Gastroenterology and Hepatology, Shaikh Zayed Hospital/ PGMI, Lahore*
[2]*Senior Registrar Gastroenterology and Hepatology, PGMI/Shaikh Zayed Medical Complex, Lahore*
[3]*Senior Registrar Gastroenterology and Hepatology PGMI/Lahore General Hospital, Lahore*
[4]*Assistant Professor, Pir Abdul Qadir Shah Jillani Institute of Medical Sciences, Gambat Khairpur Mirs Sindh*
Corresponding author: Muhammad Azhar Waseem, Email: azharwaseem818@ gmail.com, Cell: +92 302 6997435

ABSTRACT

Introduction: NAFLD is diagnosed by non-invasive and invasive methods. Non-invasive methods include NAFLD fibrosis score, AST/Platelets ratio index (APRI), FIB-4 score, BARD score, USG abdomen, Fibro-scan liver, transient elastography, MRI and MRI with elastography (MRE).

Objectives: To determine the frequency of different grades of fibrosis on shear wave elastography in patients with NAFLD and to compare mean NAFLD fibrosis score in different stages of liver fibrosis.

Study design: Cross-sectional study.

Study duration: 26th February 2021 to 25th August 2021.

Settings: Department of Gastroenterology SZH, Lahore.

Materials & Methods: A total of 197 patients with NAFLD of age 18-70 years were included. Patients with hepatitis B and/or C infection, hemochromatosis, Wilson's disease, Alpha 1 antitrypsin deficiency, celiac disease, autoimmune hepatitis, primary biliary cholangitis, primary sclerosing cholangitis were excluded. Hepatic ultrasonographic examination was performed. Blood samples were taken in a 10cc BD syringe and were sent for assessment of PT/INR, LFTs including albumin, platelets count and fasting or random glucose level. Reports were assessed and the NAFLD fibrosis scores were calculated. Shearwave elastography was used as gold standard test for the detection and confirmation of liver fibrosis; and results were compared.

Results: Age range in this study was from 18 to 70 years with mean age of 49.30 ± 12.05 years. Majority of the patients 145 (73.60%) were between 41 to 70 years of age. Out of these 197 patients, 80 (40.61%) were males and 117 (59.39%) were females with male to female ratio of 1:1.5. In this study, frequency of different grades of fibrosis on shear wave elastography in patients with NAFLD was as follows; F0 in 44 (22.34%), F1 in 89 (45.18%), F2 in 31 (15.74%), F3 in 22 (11.17%) and F4 in 11 (5.58%) patients.

Conclusion: This study concluded that non-invasive NAFLD fibrosis score should be used to rule out the presence or absence of liver fibrosis by using simple clinical and biochemical variables and thus avoid the need of liver biopsy.

Keywords: Non-Alcoholic Fatty Liver Disease, Fibrosis Score, Shearwave Elastography

INTRODUCTION

Non-alcoholic fatty liver disease is characterized by "excessive hepatic fat accumulation" and it is an increasing public health problem. NAFLD is described as "liver fat content greater than 5-10% of liver weight without alcohol intake or other cause of liver steatosis".[1] NAFLD is now considered "the hepatic manifestation of syndrome x; and insulin resistance is a key factor for the pathogenesis of both NAFLD and metabolic syndrome".[2] Though exact mechanism is still unknown but there is increased level of insulin and free fatty acids due to insulin resistance initiating complex metabolic pathways in patients with this disorder. The increased leptin and decreased adiponectin levels are also observed. Possible genetic, hormonal and nutritional factors are responsible.[3] In Western world, NAFLD affects 17-46% of adult population and its prevalence in the United States is around 30%.[4] The expected prevalence is seemed to rise in most of the developed nations given the epidemic of its major underlying determinants obesity, diabetes mellitus and metabolic syndrome. NAFLD also has increased in epidemic proportions among South Asians population like prevalence of NAFLD in China and Japan is 15% and 14% respectively. The prevalence of NAFLD in rural and urban areas in Pakistan was 9-27% and 21-42% respectively reflecting the effects of industrialization and urbanization. In one study, the prevalence of NAFLD in Pakistani population was found 72.4%.[5]

According to AASLD guidelines, Patients with NAFLD are "at increased risk of steatohepatitis, advanced fibrosis, cirrhosis and hepatocellular carcinoma". Treatment options available are weight reduction, lipid lowering medications, insulin sensitizers and anti-oxidant/anti-apoptotic medications.[6] NAFLD is diagnosed by non-invasive and invasive methods. Non-invasive methods include NAFLD fibrosis score, AST/Platelets ratio index (APRI), FIB-4 score, BARD score, USG abdomen, Fibro-scan liver, transient elastography, MRI and MRI with elastography (MRE).[7] Transient elastography has a potential to identify hepatic fibrosis and cirrhosis with a success rate of 75-92%. Invasive methods include liver biopsy by various routes. In a study, "the NAFLD fibrosis score had a potential of 52% to rule out advanced fibrosis without liver biopsy with a high negative predictive value (>92%) and average positive predictive value (79%)".[8] In another study, the sensitivity and specificity of NAFLD fibrosis score in diagnosing fibrosis was 72% and 70% respectively.[9] Similar study has shown the sensitivity and specificity of NAFLD

fibrosis score in diagnosing fibrosis was 90% and 93% respectively.[9]

Liver biopsy is weighed as the "gold standard" method to diagnose fatty liver disease. Its value in revealing the relationship between inflammation and fibrosis and excluding other causes should not be underestimated. However, certain limitations to biopsy also exist. In many studies, pain is a troublesome problem in 20% and severe complications mentioned approximately in 0.57% of cases who underwent liver biopsy procedure. The biopsy specimen may represent only 1/50,000th of the total liver size, and sampling error has been shown to be a problem in these patients.[10] Liver biopsies are not perfect to diagnose and monitor NAFLD because of invasive procedure, non-conclusive results due to sampling error and life threatening bleeding risks. So, there is intense need to develop noninvasive marker that can accurately detect advanced fibrosis.

The provisional diagnosis of NAFLD is the one of main cause for referral to hepatologist. In past, confirmation of advanced disease was based on liver biopsy. As it is not possible to biopsy each and every patient with suspected NAFLD, patients are often ranked according to priorities. Stratification of patients with fibrosis in NAFLD may be important by reason of its prognostic significance and emphasis for patients to change their lifestyle; and clinicians to check the response to treatment and standardize the treatment regimens.[11-14] To overcome these issues, we have tested scoring system to find the accuracy of NAFLD fibrosis score.

MATERIALS AND METHODS

This cross-sectional study was conducted at Department of Gastroenterology Shaikh Zayed Hospital, Lahore, during from 26th February 2021 to 25th August 2021. Total 197 patients with NAFLD of either gender were included. Patients' ages were ranging between 18 to 70 years. Patients with hepatitis B , C, alcohol, autoimmune hepatitis, primary biliary cholangitis, primary sclerosing cholangitis, haemochromatosis, Wilson's disease, Alpha 1 antitrypsin deficiency, celiac disease, drugs induced liver injury (Pyrazinamide, Paracetamol, Isoniazid, Rifampicin and others), and patients with biliary tract obstructive disease or surgical history of hepatobiliary tract were excluded.

All patients were asked about their basic demographic information like age, gender, contact details, height, weight and diabetes mellitus status. BMI was calculated by formula weight in kg/height in m2. Blood samples were taken in a 10cc BD syringe and were sent for assessment of PT/INR, LFTs including albumin, platelets count and fasting or random glucose level. Reports were assessed and the NAFLD fibrosis scores were calculated. Shearwave elastography was used as gold standard test for the detection and confirmation of liver fibrosis (as per operational definition); and results were compared.

Collected data was analyzed through computer software SPSS 25. Age, duration of NAFLD, BMI and NAFLD fibrosis score were presented as mean and standard deviation. Gender, place of living (rural/urban) and liver fibrosis on NAFLD fibrosis score were presented as frequency and percentage. Independent t- test was applied to compare the mean NAFLD fibrosis score in different stages of liver fibrosis and p-value ≤0.05 was taken as significant.

RESULTS

Age range in this study was from 18 to 70 years with mean age of 49.30 ± 12.05 years. Majority of the patients 145 (73.60%) were between 41 to 70 years of age as shown in Table I.

Out of these 197 patients, 80 (40.61%) were males and 117 (59.39%) were females with male to female ratio of 1:1.5 (Table II). Mean duration of NAFLD was 8.50 ± 3.09 months (Table III).Distribution of patients according to place of living is shown in Figure I. Mean BMI was 28.95 ± 3.28 kg/m^2.

In this study, frequency of different grades of fibrosis on shear wave elastography in patients with NAFLD was as follows; F0 in 44 (22.34%), F1 in 89 (45.18%), F2 in 31 (15.74%), F3 in 22 (11.17%) and F4 in 11 (5.58%) patients as shown in Table IV. Comparison of mean NAFLD fibrosis score in different stages of liver fibrosis is shown in Table V.

Table 1: Age distribution of patients (n=197).

Age (in years)	No. of Patients	%age
18-40	52	26.3
41-70	145	73.6
Total	197	100

Table 2: Distribution of patients according to gender (n=197).

Gender	No. of Patients	%age
Male	80	40.61
Female	117	59.39
Total	197	100

Table 3: Distribution of patients according to duration of NAFLD (n=197)

Duration (months)	No. of Patients	%age
≤6	56	28.43
>6	141	71.57

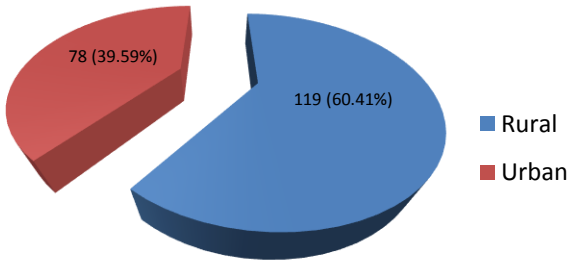

Figure 1: Distribution of patients according to place of living (n=197).

Table 4: Frequency of different grades of fibrosis on shear wave elastography in patients with NAFLD

Different grades of fibrosis	Frequency	Percentage
F0	44	22.34
F1	89	45.18
F2	31	15.74
F3	22	11.17
F4	11	5.58

Table 5: Comparison of mean NAFLD fibrosis score in different stages of liver fibrosis

Different stages of liver fibrosis	NAFLD fibrosis score		P-value
	Mean	SD	
F0	-1.536	0.189	0.0001
F1	-0.879	0.239	
F2	0.231	0.107	
F3	0.662	0.087	
F4	0.726	0.056	

DISCUSSION

The limitations of liver biopsy have driven a search for non-invasive NAFLD screening and risk stratification methods. Since advanced fibrosis has been proven to be prognostic of poor outcomes in NAFLD, multiple surrogate fibrosis markers have been studied, including clinical predictors, serum biomarkers, and imaging methods.[124] One of these methods, the NAFLD fibrosis score is used to assess advanced fibrosis risk. In this method, clinical parameters such as age, body mass index, albumin, AST/ALT ratio, etc., are used to calculate a score. A score of > 0.676 has an 82% positive predictive value in diagnosing advanced liver fibrosis (stage ≥ 3 in a 5-stage fibrosis scoring system) in patients with histology-proven NAFLD [15]. Serum biomarkers such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) have been shown to be elevated in patients with NAFLD/NASH, although normal aminotransferase levels do not exclude the diagnosis of SS or NASH; patients with advanced NAFLD have been reported to have normal ALT levels [16].

A variety of imaging modalities are increasingly used for NAFLD evaluation and include conventional imaging techniques as well as newer technologies. Conventional imaging techniques consist of B-mode ultrasonography (US), computed tomography (CT), and magnetic resonance (MR) imaging. Findings in NAFLD patients with these techniques are based on lipid accumulation. However, evaluation of inflammation and degrees of fibrosis less than cirrhosis are not possible with conventional imaging techniques. Newer imaging technologies are being increasingly used in combination with conventional technologies and include ultrasound elastography (USE), quantitative ultrasound-based techniques, magnetic resonance elastography (MRE), and magnetic resonance-based fat quantitation techniques [17].

Fibroscan employs ultrasound transient elastography (TE) to measure hepatic elasticity by quantifying the shearwave speed with pulse-echo ultrasound from low frequency vibrations that are transmitted into the liver [18]. It is able to detect liver cirrhosis with high accuracy, and liver stiffness measurements correlate with liver fibrosis stages [19]. In a NAFLD study with 246 subjects, the AUROCs for the detection of F ≥ 2 and F ≥ 3 were 0.84 and 0.93, respectively, and the sensitivity and specificity for F ≥ 3 were 91% and 75% at a cutoff value of 7.9 kPa [20]. A lower TE value appears to reliably exclude advanced fibrosis [21]. Because transient elastography requires transmission of a mechanical wave that originates at the skin, obesity is a significant cause of technical failure and unreliable measurements. To address this problem, the Fibroscan XL probe was developed for obese patients. Controlled attenuation parameter (CAP) is another technique implemented on the Fibroscan device. The reduction in ultrasound amplitude can be estimated as the sound wave traverses liver tissues using the same radiofrequency [22]. In a study of 183 patients, CAP showed good capability in discriminating NASH from simple steatosis, with an AUROC of 0.812 (95%CI: 0.724-0.880) [23].

A retrospective meta-analysis, evaluating 2D-SWE in 1340 patients with chronic liver diseases from 13 centers worldwide, reported diagnostic accuracies of 91% and 95% for advanced fibrosis and cirrhosis, and optimal cutoffs of 9.2, and 13.5 kPa, respectively [24]. In the subgroup of 172 NAFLD patients, diagnostic accuracies were 93% and 92% for advanced fibrosis and cirrhosis, respectively, with the same optimal cutoffs as for the overall group. When 2D-SWE was compared to TE in a subgroup of 91 NAFLD patients with reliable TE-values, 2D-SWE performed significantly better for diagnosing advanced fibrosis (AUROC difference of 12%; P = .003). In another study in 291 NAFLD patients, 2D-SWE had diagnostic accuracies of 89% and 88% for detecting advanced fibrosis and cirrhosis, respectively [25].

Cassinotto et al. performed the only study to evaluate SWE in NAFLD patients [26]. Overall, SWE had statistically better diagnostic performance for fibrosis stage ≥2 than pSWE with AUROC of 0.85 versus 0.76 (P = 0.004), but similar diagnostic accuracy to VCTE (AUROC, 0.85 vs. 0.83; P = 0.5). LSM failures occurred in 15% of patients, whereas unreliable results occurred in 7.2% of cases. These rates are very similar to VCTE, with obesity having the greatest impact. In fact, reliable results were obtained in approximately 90% of patients with a BMI <30 kg/m2, but only 73% of patients with a BMI ≥30 kg/m2. Optimal LSM cutoff values for SWE were: fibrosis stage ≥ 2, 6.3 to 8.7 kPa, with 71% to 90% sensitivity, 50% to 90% specificity, and AUROC 0.79 to 0.90; fibrosis stage ≥ 3, 8.3 to 10.7 kPa, with 71% to 91% sensitivity, 71% to 90% specificity, and AUROC 0.83 to 0.92; fibrosis stage 4, 10.5 to 14.4 kPa, with 58% to 90% sensitivity, 72% to 90% specificity, and AUROC 0.82 to 0.92 [26].

The NFS is arguably the most studied scoring system and is recommended by the American Association for the Study of Liver Diseases (2012) [27] and European Association for the Study of the Liver (2015) [28] in the assessment of patients for advanced fibrosis. In comparison to other composite scores for advanced fibrosis, the NFS was found to perform favorably [29]. The NFS was developed in a multicenter study of 733 patients with biopsy-proven NAFLD [30]. Four-hundred-and-eighty patients were used to develop the scoring system, with the remaining two-hundred-and-fifty-three patients used for validation. Six variables including age, hyperglycemia, body mass index (BMI), platelet count, albumin and the AST/ALT ratio were identified to be independent indicators of

advanced fibrosis. Using these variables, a regression formula was applied to create the NFS score. Two optimal cut-offs were identified, one to exclude advanced fibrosis (<−1.455) and the other to indicate the presence of advanced fibrosis (>0.676). Using these cut-offs, the NFS score was able to discriminate patients with advanced fibrosis (stage ≥3) from patients without (stage 0–2), with an area under the receiver operating characteristic curve (AUROC) of 0.82 (95% CI 0.76–0.88) in the validation cohort [30].

The NFS score was validated in another study comparing various non-invasive measures of advanced fibrosis, with an AUROC of 0.81 (95% CI, 0.71–0.91), performing well when compared with the FIB-4, BARD score, and AST/ALT ratio [29] In a meta-analysis of 13 studies consisting of 3,064 patients [31], the NFS had an AUROC of 0.85 for predicting advanced fibrosis (F ≥ 3). A score of <−1.455 had 90% sensitivity and 60% specificity for excluding advanced fibrosis, whereas a score of >0.676 had 67% sensitivity and 97% specificity for identifying the presence of advanced fibrosis. While the NFS represents an easily accessible tool, incorporating routine clinical parameters, and has good diagnostic performance, a considerable proportion of patients (between 20–58%) do fall into the indeterminate “grey zone” [31].

CONCLUSION

This study concluded that frequency of different grades of fibrosis on shear wave elastography in patients with NAFLD was as follows; F0 in 22.34%, F1 in 45.18%, F2 in 15.74%, F3 in 11.17% and F4 in 5.58% patients with mean NAFLD fibrosis score increases as the grade of fibrosis increases. So, we recommend that non-invasive NAFLD fibrosis score should be used to rule out the presence or absence of liver fibrosis by using simple clinical and biochemical variables and thus avoid the need of liver biopsy.

REFERENCES

1. EASL–EASD–EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. J Hepatol. 2016 Jun;64(6):1388-402.
2. Li Q, Dhyani M, Grajo JR, Sirlin C, Samir AE. Current status of imaging in nonalcoholic fatty liver disease. World J Hepatol. 2018;10(8):530–42.
3. Pappachan JM, Babu S, Krishnan B, Ravindra NC. Non-alcoholic fatty liver disease: a clinical update. J Clin Transl Hepatol. 2017;5(4):384–93.
4. Rinella ME. Nonalcoholic fatty liver disease: a systematic review. JAMA. 2015;313:2263–2273.
5. Pati GK, Singh SP. Nonalcoholic Fatty Liver Disease in South Asia. Euroasian J Hepato-Gastroenterol. 2016;6(2):154-62.
6. Chalasani N, Younossi Z, Lavine JE, Charlton M. The diagnosis and management of nonalcoholic fatty liver disease: practice guidance from the american association for the study of liver diseases. Hepatol. 2018;67(1):328-57.
7. Feldman M, Friedman LS, Brandt LJ. Sleisenger and Fordtran’s Gastrointestinal and Liver Disease. Elsevier saunders 2016;2(10):1429-441.
8. McPherson S, Stewart SF, Henderson E. Simple non-invasive fibrosis scoring systems can reliably exclude advanced fibrosis in patients with non-alcoholic fatty liver disease. Gut. 2010;59:1265-69.
9. Xiao G, Zhu S, Xiao X, Yan L, Yang J, Wu G. Comparison of laboratory tests, ultrasound, or magnetic resonance elastography to detect fibrosis in patients with nonalcoholic fatty liver disease: A meta-analysis. Hepatology. 2017 Nov;66(5):1486-1501.
10. Jiang W, Huang S, Teng H, et al. Diagnostic accuracy of point shear wave elastography and transient elastography for staging hepatic fibrosis in patients with nonalcoholic fatty liver disease: a meta-analysis. BMJ Open. 2018;8:e021787.
11. Treeprasertsuk S, Bjornsson E, Enders F. NAFLD fibrosis score: a prognostic predictor for mortality and liver complications among NAFLD patients. World J Gastroenterol. 2013;19(8):1219-29.
12. Parkash O, Hamid S. Are we ready for a new epidemic of under recognized liver disease in South Asia especially in Pakistan? Non-alcoholic fatty liver disease. J Pak Med Assoc. 2013;63(1):95-9.
13. GIHEP. Resources for Gastroenterology & Hepatology. Available from http://gihep.com/calculators/hepatology/.
14. Angulo P, Bugianesi E, Bjornsson ES. Simple non-invasive systems predict long term outcomes of patients with non-alcoholic fatty liver disease. J Gastro. 2013;145(4):782-9.
15. Angulo P, Hui JM, Marchesini G, Bugianesi E, George J, Farrell GC, Enders F, Saksena S, Burt AD, Bida JP, et al. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. Hepatology. 2007;45:846–854.
16. Ballestri S, Nascimbeni F, Romagnoli D, Lonardo A. The independent predictors of non-alcoholic steatohepatitis and its individual histological features.: Insulin resistance, serum uric acid, metabolic syndrome, alanine aminotransferase and serum total cholesterol are a clue to pathogenesis and candidate targets for treatment. Hepatol Res. 2016;46:1074–1087.
17. Fargion S, Porzio M, Fracanzani AL. Nonalcoholic fatty liver disease and vascular disease: state-of-the-art. World J Gastroenterol. 2014;20:13306–13324.
18. Yoneda M, Suzuki K, Kato S, Fujita K, Nozaki Y, Hosono K, Saito S, Nakajima A. Nonalcoholic fatty liver disease: US-based acoustic radiation force impulse elastography. Radiology. 2010;256:640–647.
19. Foucher J, Chanteloup E, Vergniol J, Castéra L, Le Bail B, Adhoute X, Bertet J, Couzigou P, de Lédinghen V. Diagnosis of cirrhosis by transient elastography (FibroScan): a prospective study. Gut. 2006;55:403–408.
20. Friedrich-Rust M, Wunder K, Kriener S, Sotoudeh F, Richter S, Bojunga J, Herrmann E, Poynard T, Dietrich CF, Vermehren J, et al. Liver fibrosis in viral hepatitis: noninvasive assessment with acoustic radiation force impulse imaging versus transient elastography. Radiology. 2009;252:595–604.
21. Dyson JK, McPherson S, Anstee QM. Republished: Non-alcoholic fatty liver disease: non-invasive investigation and risk stratification. Postgrad Med J. 2014;90:254–266.
22. Chan WK, Nik Mustapha NR, Mahadeva S. Controlled attenuation parameter for the detection and quantification of hepatic steatosis in nonalcoholic fatty liver disease. J Gastroenterol Hepatol. 2014;29:1470–1476.
23. Lee HW, Park SY, Kim SU, Jang JY, Park H, Kim JK, Lee CK, Chon YE, Han KH. Discrimination of Nonalcoholic Steatohepatitis Using Transient Elastography in Patients with Nonalcoholic Fatty Liver Disease. PLoS One. 2016;11:e0157358.
24. Herrmann E, de Lédinghen V, Cassinotto C. Assessment of biopsy-proven liver fibrosis by two-dimensional shear wave elastography: an individual patient data-based meta-analysis. Hepatology. 2018; 67: 260–272
25. Cassinotto C, Boursier J, de Lédinghen V. Liver stiffness in nonalcoholic fatty liver disease: a comparison of supersonic

shear imaging, FibroScan, and ARFI with liver biopsy. Hepatology. 2016; 63: 1817–1827.
26. Cassinotto C, Boursier J, de Ledinghen V, Lebigot J, Lapuyade B, Cales P, et al. Liver stiffness in nonalcoholic fatty liver disease: a comparison of supersonic shear imaging, FibroScan, and ARFI with liver biopsy. Hepatology 2017; 63: 1817– 1827.
27. Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, et al. The diagnosis and management of non-alcoholic fatty liver disease: practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. Hepatology. 2012;55:2005–2023.
28. European Association for Study of Liver; Asociacion Latinoamericana para el Estudio del Higado EASL-ALEH Clinical Practice Guidelines: Non-invasive tests for evaluation of liver disease severity and prognosis. J. Hepatol. 2015;63:237–264.
29. McPherson S, Stewart SF, Henderson E, Burt AD, Day CP. Simple non-invasive fibrosis scoring systems can reliably exclude advanced fibrosis in patients with non-alcoholic fatty liver disease. Gut. 2010;59:1265–1269.
30. Angulo P, Hui JM, Marchesini G, Bugianesi E, George J, Farrell GC, et al. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. Hepatology. 2007;45:846–854.
31. Musso G, Gambino R, Cassader M, Pagano G. Meta-analysis: natural history of non-alcoholic fatty liver disease (NAFLD) and diagnostic accuracy of non-invasive tests for liver disease severity. Ann Med. 2011;43:617–649.