Histological Evaluation of Non-alcoholic Fatty Liver Disease and Its Correlation with Different Noninvasive Scoring Systems with Special Reference to Fibrosis: A Single Center Experience

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Background: **Although liver biopsy remains the gold standard for the diagnosis of non-alcoholic fatty liver disease [NAFLD], many non-invasive markers of liver fibrosis have recently been proposed and assessed as surrogates of liver biopsy.** ***Aims and objective:*** **To evaluate the degree of liver fibrosis by different non-invasive fibrosis scoring systems and to compare each non-invasive fibrosis scoring system with histological fibrosis stage.** ***Materials and methods:*** **The study population consists of consecutive patients with biopsy proven NAFLD. Complete medical history was taken and physical examination was done in all patients along with appropriate biochemical evaluations. NAFLD fibrosis score, BARD score, BAAT score and APRI score were calculated and each score was compared with histological fibrosis staging.** ***Results:*** **The study population consisted of 60 patients having mean age 39.73 years (SD 9.62, range 17–63 years) including 51 (85%) males and 9 (15%) females. On histology fibrosis was present in 68.3% (41/60) patients. Out of 60 patients 41 had fibrosis and among them 17, 22, 2 patients had grade 1, 2, 3 fibrosis respectively and no one had grade 4 fibrosis. 61.67% (37/60) had definite NASH. Comparing the fibrosis of histology with the noninvasive scoring systems, the sensitivity and specificity of NAFLD fibrosis score were 5.56% and 100% respectively. BARD score had 45.83% sensitivity and 80.55% specificity. The sensitivities of BAAT score and APRI score were 0% and 29.16% respectively and the specificities were 100% and 97.22% respectively.** ***Conclusion:*** **The noninvasive scoring systems like NFS, BARD, BAAT, and APRI are not sensitive enough to detect fibrosis but highly specific to include fibrosis if scores are more than cut-off values in our cohort, however they cannot replace liver biopsy. Newer more efficient non-invasive scoring systems have to be devised for the Indian NAFLD population.** **(J Clin Exp Hepatol 2016;6:291–296)**

Non-alcoholic fatty liver disease (NAFLD) is now considered to be the commonest cause of chronic liver disease in both developed as well as developing countries. NAFLD has become a major public health problem due to the rising prevalence of obesity and T2DM worldwide.[1] The prevalence of NAFLD is between 20% to 40% in the general population in the West[2,3] while it varies from 8% to 40% in India.[4–7] Although NAFLD was earlier considered to be a relatively benign condition, up to a third of patients may develop serious consequences, including end-stage liver disease and hepatocellular carcinoma.[8–11] Those at risk are patients with significant hepatic necroinflammation and fibrosis.[12–14]

Liver biopsy is currently the gold standard, and has been recommended for confirming the diagnosis and for providing prognostic information in cases of NAFLD.[15] The procedure is invasive and prone to complications, with 0.01% risk of death.[16,17] It also has the limitations of sampling error and inter-observer variability.[18] As there is high prevalence of NAFLD in our population,[4,19] liver biopsy may not be a logistically feasible option in all. In view of these limitations and incumbent risks, and the high prevalence of the disease and grossly inadequate population of hepatologists who can perform liver biopsy, we need non-invasive tests that can reliably diagnose or

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Abbreviations: ALT: alanine aminotransferase; APRI: aspartate aminotransferase (AST)-to-platelet ratio index; AST: aspartate aminotransferase; BMI: body mass index; DM: diabetes mellitus; NAFLD: non-alcoholic fatty liver disease; NASH: non-alcoholic steatohepatitis; NAS: NAFLD fibrosis score; NPV: negative predictive value; PPV: positive predictive value; ROC: receiver operating characteristic; SGOT: serum glutamic oxaloacetic transaminases; SGPT: serum glutamic pyruvate transaminases; TPC: total platelet count; TG: triglyceride
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exclude significant fibrosis, which would be clinically useful to reduce the need for liver biopsy and to prognosticate patients. Currently several clinical scoring systems based on simple clinical or laboratory indices have been proposed to identify advanced fibrosis in patients with NAFLD and other liver diseases. These include the aspartate aminotransferase (AST)-to-platelet ratio index (APRI),[20] the aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ratio,[21] the BARD[BMI, AST/ALT, DM] score,[22] the BAAT[BMI, Age, ALT, TG] score,[23] the FIB-4[Age, AST, ALT, Platelets] score[24] and the NAFLD fibrosis score (NAS).[25] However, most of them need to be validated prior to their use in any setting. The present study was undertaken to evaluate the efficacy of some of these noninvasive scoring systems in diagnosing fibrosis in NAFLD patients from coastal eastern India.

METHODS

In this observational study, conducted at Cuttack, Odisha from July 2011 to September 2013, 60 consecutive outpatients who had fatty liver on ultrasonography, and biopsy proven NAFLD were included. Ultrasonography was performed using a curvilinear 3–5 mHz probe (Philips) by two experienced sonologists. Men who consumed >20 g and women consuming >10 g of alcohol per day, patients with evidence of concomitant liver diseases, hemolysis, Gilbert's disease, HIV infection or on immunosuppressive therapy were excluded from the study. Serum HBsAg, anti-HCV, HIV, ceruloplasmin by copper oxidase method, antinuclear antibody, anti-smooth muscle antibody, anti-LKM antibody, serum protein electrophoresis, urinary copper and KF ring on slit-lamp examination were done wherever indicated to exclude other causes of liver diseases. Written informed consent was obtained from all the subjects. The protocol was approved by the institutional ethics committee.

Anthropometric parameters like height, weight, body mass index (BMI), waist and hip circumferences and waist/hip ratio were recorded in all study subjects. All patients were subjected to hematological workup including complete blood count and biochemical investigations like liver function test, lipid profile and fasting blood glucose. All biochemical assessments were performed in the same laboratory by standard laboratory methods. FBG and lipid profile were assayed by an autoanalyser (BIOLIs 24i Tokyo Boeki, Japan) using standard kit. Liver biopsy was performed after informed consent in 60 patients who agreed for it by using a 16 gauge automated cutting biopsy gun (BARD, USA) through the intercostal approach under ultrasound guidance. Tissue obtained at biopsy were fixed in formalin and subjected to routine paraffin embedding and Hematoxylin and Eosin (H&E) staining, ensuring adequate sections to examine the entire biopsy. Besides H and E staining, sections were also stained with special stains, Reticulin and Masson's trichrome. Histological confirmation of NAFLD was done by using the NAFLD activity score. The histological score was defined as the unweighted sum of the scores for steatosis (0–3), lobular inflammation (0–3), and ballooning (0–2). The patients were categorized into three groups: definite NASH, borderline NASH and 'not NASH' on the basis of modified Kleiner et al.'s[26] NAS [NAFLD activity score] criteria, wherein they have classified histology of hepatic steatosis into NASH with a NAS of $\geq$5, and No NASH when NAS was less than 3. We labeled the histologies having intermediate scores of 3 and 4 as borderline NASH in our study. Specimens were evaluated by a two pathologists for necro-inflammation and fibrosis as per the NAS-II system. Significant fibrosis was defined as fibrosis $\geq$2 and minimal fibrosis as F0 or F1. The potential markers of fibrosis were then correlated with liver biopsy findings of necro-inflammation and fibrosis.

Non-invasive scoring systems like NAFLD fibrosis score [$-1.675 + 0.037 \times$ age (years) $+ 0.094 \times$ BMI (kg/m^2) $+ 1.13 \times$ IFG/diabetes (yes = 1, no = 0) $+ 0.99 \times$ AST/ALT ratio $- 0.013 \times$ platelet ($\times 10^9$/L) $- 0.66 \times$ albumin (g/dl)],[25] BARD score [BMI, AST/ALT,DM],[22] BAAT score [BMI, Age, ALT, TG][23]and APRI [AST/upper limit of normal $\times$ 100/platelet count ($\times$109/L)][20] were calculated and compared. There is significant fibrosis when NFS > 0.676, APRI > 0.88, BARD $\geq$ 2 and if BAAT score is 0 or 1, then NPV for advanced fibrosis is 100%.

Statistical Methods

Data were checked for normal distribution using Shapiro–Wilk test. Categorical and continuous data were presented as proportion and mean, standard deviation and 95% confidence intervals, respectively. Chi-square and unpaired *t* tests were used to compare between categorical and continuous data, respectively. *P*-values below 0.05 were considered significant for all statistical analysis. Statistical analysis was done using SPSS version 16 (SPSS, Inc., Chicago, IL, USA). Sensitivity and specificity for each clinical score were computed and the values obtained were plotted on a ROC (receiver operating characteristic) curve.

RESULTS

The 60 patients with NAFLD had mean age 39.73 years (SD 9.62; range 17–63) and included 51 (85%) males and 9 (15%) females with a gender ratio of 5.67:1. Most patients were in the 4th and 5th decades of life as shown in Table 1. In this cohort, 10 (16.67%) patients had diabetes, 16 (26.67%) patients had hypertension and 41 (68.33%) patients had obesity. Their clinical and biochemical parameters are shown in Table 2.

Among the 60 patients with NAFLD, 8 (13.33%) patients had no NASH, 15 (25%) had borderline NASH