Research Article

Assessment of Liver Fibrosis Grading with Non Invasive Scoring Systems Among Non Alcoholic Fatty Liver Disease (NAFLD) Patients

Sabhita Shabir Shaikh,[1] Saba Nafay,[2] Shirin Jalauddin Reshamwala,[3] Sidra Zaheer,[4] Madiha Sajid[5]

[1,2]National Institute of Liver and GI Diseases (NILGID), Dow University Hospital Karachi Pakistan; [3]Aga Khan University, Karachi; [4]School of Public Health, Dow University Hospital Karachi Pakistan; [5]Department of Dermatology, Dow University Hospital Karachi Pakistan

Abstract

Background: The evaluation of degree of liver fibrosis is an important prognostic factor in the management of NAFLD patients. Noninvasive risk stratification is needed to triage individuals with advanced fibrosis for optimal treatment and surveillance.

Objective: To find the frequency of fibrosis in NAFLD patients using multiple non-invasive tests and to match the performance of noninvasive tests in diagnosing liver fibrosis.

Methods: An observational study done in Out-patient Clinics of National Institute of Liver and GI diseases (NILGID), Dow University Hospital from January 2021 to January 2022. All consecutive NAFLD patients during the study period were enrolled. Liver fibrosis was assessed in each patient with non invasive scoring including APRI score (AST to platelet ratio index), AST/ALT ratio, NFS (NAFLD fibrosis scores) and FIB-4.

Results: 322 patients (mean age 44.84 ±10.94 years) were included. The mean values for ALT and AST levels were 40.67 ± 31.69IU/L and 31.85 ± 22.60 respectively. The best diagnostic accuracy was observed with FIB4 score having AUROC of 0.904. A robust correlation was observed between APRI index and FIB-4 score (r = 0.734, p-value < 0.01), and between FIB-4 score and NFS score (r = 0.700, p- value <0.01). Whereas correlation of APRI index with NFS score was moderate (r=0.394), and with AST/ALT ratio was weak (r = 0.201).

Conclusion: Noninvasive markers, FIB4 and NFS score demonstrate a dependable ability to diagnose and rule out liver fibrosis, which proves to be an economical approach in management of patients with NAFLD.

Corresponding Author | Dr Sabhita Shabir Shaikh, Assistant Professor, National Institute of Liver and GI Diseases (NILGID), Dow University Hospital Karachi Pakistan; Aga Khan University, Karachi. **Email:** sabhita.shabbir@gmail.com

Keywords | NAFLD, fibrosis, FIB4, diagnosis, non-invasive, NFS, APRI.

Introduction

Nonalcoholic fatty liver disease (NAFLD) was first considered in the early 1980s as an unnamed condition that most closely resembled alcoholic hepatitis and could cause cirrhosis.[1] In the years since, NAFLD has been defined as one of the most pervasive causes of hepatic pathology, strongly associated with obesity and diabetes. While the most common presentation of this disease is patients presenting with simple steatosis, studies have shown that up to half of all patients with NAFLD will develop complications that may include liver fibrosis and cirrhosis, and may culminate in either hepatocellular carcinoma (HCC) or end stage liver failure.

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Over the years following its discovery, the incidence of NAFLD has consistently increased. With the escalating rates of obesity, there are predictions that NAFLD might soon emerge as the primary cause of liver disease on a global scale. Based on current literature, it is estimated that around 37% of the universal adult population is influenced by NAFLD.[2] The distribution varies, with higher rates reported in the United States and Middle East as compared to Africa. In Western countries, the overall frequency of NAFLD varies from 15% to 40%, while in Asian countries, it ranges from 9% to 40%. As of 2020, the probable total prevalence of NAFLD in Asian countries is 29.6%, indicating that it may have already exceeded the figures seen in Western countries.[3] One of the most recent NAFLD studies done in Pakistan gives a likely prevalence of 15% in the overall population.[4]

NAFLD is often largely asymptomatic and often been described as an indolent disease. This in turn often leads patients to either miss the early signs or misperceive the danger of a condition that is closely linked to metabolic syndrome and type 2 diabetes mellitus. In addition, cardiovascular events have been identified as the most common cause of mortality in patients with NAFLD, followed by cirrhosis and hepatocellular carcinoma.[5] In light of such grave consequences, there is an onus on the medical community to correctly diagnose the presence of steatohepatitis and fibrosis in this population as the disease progression and prognosis depends largely on it. Incorrect perceptions about medical conditions may lead to detrimental decisions being taken by and for patients.

Assessment of liver fibrosis should be carried out among all patients with NAFLD which will predict the threat of upcoming development of hepatic complications and hence needed for management decisions, observing the disease progression and planning for surveillance strategies.

The noninvasive tests (NITs) are being established as cost controlled screening tools for risk stratification of patients for the development of advanced liver fibrosis. Scores like Fibrosis-4 Index (Fib-4), NAFLD Fibrosis Score (NFS), the ratio of the serum aspartate to alanine amino-transferase levels (AST/ALT), and aspartate aminotransferase to platelet ratio (APRI) have been deployed across various populations. NITs overwhelmed many restrictions of liver biopsies being performed and thus are now regularly integrated into expert medical practice.[6]

The sensitivity and specificity of these noninvasive markers has varied over different populations. Singh et al.[7] found that the APRI score with cutoff values of >1.5 had 94.7% specificity and 16.5 % sensitivity, NFS score with cutoff values of >0.676 had 69.9 % specificity and 63.7 % sensitivity while FIB-4 score with cutoff values of >2.67 had 93 % specificity and 44% sensitivit. In comparison, McPherson8reported sensitivity and specificity for the FIB-4 score were 85% and 65% respectively, for the NFS score, 78% sensitivity and 58% specificity and APRI index sensitivity was 27% was specificity was 89%. A ratio >0.8 of AST: ALT is a marker of more severe liver disease. Literature suggest that the sequential combinations of NITs increases sensitivity and specificity thus could minimize the need for liver biopsies.[8]

The suggested approach usually involves the sequential application of two NITs, first with the use of simple, inexpensive, serum-based tests which could be performed in the community (e.g., FIB-4 or NFS), which can be followed by a second set of measuresment of liver stiffness (LSM) by either a serum-based test (e.g., enhanced liver fibrosis test; ELF) or by transient elastography (TE).[9]

In a recent hospital based study, prevalence of NAFLD was estimated to be 75%, in a diabetic patient population in Northern Pakistan.[10] Studies like these are few and far in between. Given the previously discussed disease severity we feel it is important to fill this gap in data for the population in Pakistan.

Also owing to limited resources and lack of financial assistance, the patients in Pakistan, especially in rural areas do not have frequent hospital visits / medical checkups.

This study targets to evaluate the frequency of stage of liver fibrosis in NAFLD patients with several simple non-invasive scoring systems and to then compare the efficacy of various non-invasive biomarkers in the absence of a gold standard. So that timely intervention can be done in these underprivileged patients within limited resources.

Methods

It was an observational study in which 322 consecutive patients diagnosed with NAFLD on ultrasound, during the study period, and aged 18 to 65 years were recruited from the Out-patient Department (OPD) of National Institute of Liver and GI diseases (NILGID), Dow University Hospital from 4th January 2021 to 3rd January 2022. Ethical approval was obtained from the Dow University Institutional Review Board (IRB 1842). Sample size was calculated using OpenEpi calculator with 95% confidence interval, 5% margin of error and 20% prevalence of stage 4 fatty liver patients[10]. The calculated sample size was 246, which was raised up to 322 patients.

Laboratory and clinical data was collected during out-patient visits. Patients were omitted from the study in the presence of decompensated chronic liver disease, hepatoma/hepatocellular carcinoma, acute hepatic flare, acute on chronic liver failure or any other simultaneous liver disease or patients using alcohol or other hepatotoxic drugs. Pregnant or lactating females were also excluded from the study population as well as having systemic diseases like autoimmune diseases, tuberculosis and non hepatic malignancies.

Once written consent was obtained relevant clinical details including patient's demographics such as age, gender, marital status, height , weight and body mass index (BMI), comorbidities such as diabetes mellitus/ hypertension were gathered via structured interview based questionnaire. Laboratory investigations including serum albumin, liver function tests, fasting lipid profile and platelet count were also noted.

Then the non- invasive assessment of liver fibrosis was done for each patient. APRI, AST/ALT ratio, NFS and Fib-4 scores were calculated using online calculator.

FIB-4 panel includes age, platelet count, AST, and ALT.

Calculated from the following formula,

$$\text{Age (in years)} \times \text{AST level (in IU/L)}$$

$$\text{Platelet count } (\times 10^9/\text{L}) \times \sqrt{\text{ALT (in IU/L)}}$$

Cut off values used were as follows: FIB-4 less than 1.45 as mild, 1.45 to 3.25 as moderate as and greater than 3.25 as severe fibrosis.

NFS panel consists of platelets, albumin, age, BMI, AST/ALT ratio and hyperglycemia, it is calucuated with the formula

$$-1.675 + 0.037 \times \text{age (in years)} + 0.094 \times \text{BMI (in kg/m}^2) + 1.13 \times \text{impaired fasting glucose /diabetes (yes = 1, no=0)} + 0.99 \times \text{AST/ALT ratio} - 0.013 \times \text{platelet } (\times 10^9/\text{l}) - 0.66 \times \text{albumin (in g/dl)}$$

NFS, less than -1.455 as mild, between -1.455 to 0.676 as moderate, greater than 0.676 as severe fibrosis.

Cutoff values for APRI , was 0.5 for mild and 1.5 for advanced fibrosis respectively.

Whereas AST/ALT ratio>0.8 is taken an indicator of more severe liver disease.

Patients were consequently informed about their stage of liver fibrosis based on this scoring system and advised appropriate follow up visits accordingly.

The Statistical Package for the Social Sciences (SPSS) software, version 26 was used for data entry and analysis. Quantitative variables like clinical parameters and age were summarized using mean and standard deviation. Categorical variables such as gender and NAFLD severity were described using frequency and percentages. The normality of quantitative variables was evaluated using the Shapiro-Wilk test. Correlations between different indices were determined using Pearson's correlation coefficient. To assess the diagnostic performance of the indices for the severity of liver fibrosis, receiver operating characteristic (ROC) curves were generated. The area under the ROC curve (AUROC) and the optimal cut-off values were calculated for each non-invasive index. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy (DA) were also calculated for these cut-off values. Statistical significance was considered if P values ≤ 0.05.

Results

322 patients diagnosed with NAFLD were enrolled and analyzed in this study having mean age of 44.84 ± 10.94 years, 91 (28.3%) were males and 231 (71.7%) were females. The mean platelet counts were 295.77 ± 90.85/uL, the mean ALT levels and the AST levels were 40.67 ± 31.69IU/L and 31.85 ± 22.60 respectively. (Table 1)

For each index, fibrosis scores were computed for all 322 patients. According to the APRI index, 4 patients

Table 1: *Clinical and demographic and features of study population (n=322)*

Characteristics	n (%)
Gender	
male	91 (28.3)
female	231 (71.7)
Marital status	
Unmarried	17 (5.3)
Married	305 (94.7)
Any comorbidity (DM/HTN)	
No	192 (59.6)
Yes	130 (40.4)
	Mean ± SD
Age	44.84 ± 10.94
BMI (kg/m2)	29.83 ± 5.52
Total bilirubin (mg/dl)	0.58 ± 0.74
AST (IU/L)	31.85 ± 22.60
ALT (IU/L)	40.67 ± 31.69
GGT (IU/L)	46.02 ± 34.77
Serum Platelet (/uL)	295.77 ± 90.85
Serum Albumin (g/dl)	4.41 ± 0.38
Serum cholesterol (mg/dl)	183.29 ± 45.18
Serum Triglycerides (mg/dl)	184.79 ± 101.2
LDL (mg/dl)	124.81 ± 39.85
APRI index	0.35 ± 0.31
FIB-4 score	0.88 ± 0.67
NFS score	-2.41 ± 1.71
AST/ALT ratio	0.91 ± 0.44

(1.2%) had severe fibrosis, while 59 patients (18.3%) had moderate fibrosis. According to the FIB-4 score, 4(1.2%) patients carried a score more than 3.25, indicating very severe fibrosis, while 29 (9.0%) patients had scores between 1.45 and 3.25, indicating moderate fibrosis. Based on NFS score, 86 (26.7%) patients classified as moderate and 12 (3.7%) patients as severe fibrosis. Regarding the AST/ALT ratio, 189 (58.7%) patients had intermediate fibrosis, whereas 133 (41.3%) patients had advanced fibrosis. (Table 2)

Among different indices, a robust strong association was established between APRI index and FIB-4 score

Table 2: *Severity of NAFLD with different indices*

	NAFLD Severity		
	Mild	Moderate	Severe
	n (%)	n (%)	n (%)
APRI index	259 (80.4)	59 (18.3)	4 (1.2)
FIB-4 score	289 (89.8)	29 (9.0)	4 (1.2)
NFS score	224 (69.6)	86 (26.7)	12 (3.7)
AST/ALT ratio	189 (58.7)		133 (41.3)

(r = 0.734, p-value <0.01), and between FIB-4 score and NFS score (r = 0.700, p-value <0.01). Whereas, the correlation of APRI index with NFS score was moderate (r=0.394), with AST/ALT ratio was weak (r = 0.201). The correlation of NFS score and FIB-4 score with AST/ALT ratio was moderate (r = 0.573 and r = 0.398 respectively). (Table 3)

When using the APRI index as a reference, the diagnostic

Table 3: *Correlation coefficient between different indices*

	APRI index	FIB-4	NFS score	AST/ALT ratio
APRI index	1	0.734**	0.394**	0.201**
FIB-4		1	0.700**	0.573**
NFS score			1	0.398**
AST/ALT ratio				1

** Correlation is significant at the 0.01 level

performance of each index is mentioned in Table 4. FIB-4 score was the best measure to distinguish between severe fibrosis and mild to moderate fibrosis, when com-

Table 4: *Diagnostic performance of the indices for the risk of risk of severe liver fibrosis*

Indices	AUC (95% CI)	p-value	Optimal cut off	Sensitivity	Specificity	PPV	NPV	DA
				%	%	%	%	%
FIB-4	0.904 (0.859-0.949)	<0.001	1.190	73.5	86.8	39.6	96.5	85.4
NFS score	0.740 (0.652-0.829)	<0.001	0.675	20.6	98.3	58.3	91.1	90.0
AST/ALT ratio	0.611 (0.501-0.721)	0.048	0.940	55.9	66.6	16.5	92.7	65.5

CI confidence interval, DA diagnostic accuracy, PPV positive predictive value, NPV negative predictive value, AUC area under the curve

pared to the APRI index, , with an AUC curve of 0.904 (95% CI 0.859-0.949). For this purpose, the ideal FIB-4 cutoff score was 1.190, with a sensitivity of 73.5%, specificity of 86.8%, and DA of 85.4%.

Figure 1 displays the ROC curves of all indices for the identification of severe fibrosis.

The results showed that FIB-4 score was the best indicator when compared to NFS score and AST/ALT ratio if APRI index was used as the gold standard.

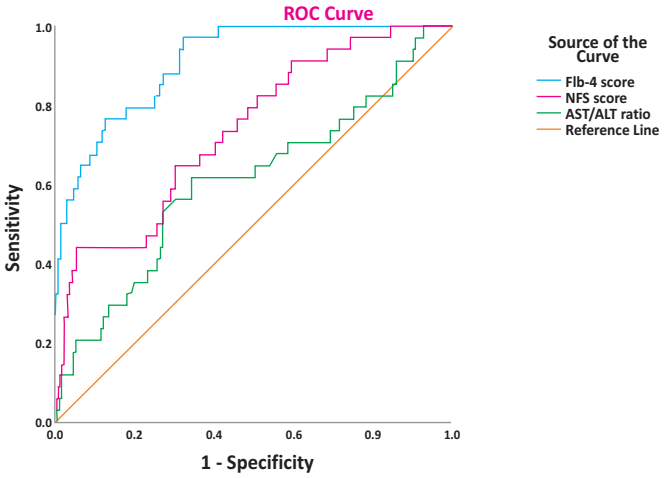

Figure1: *ROC curves of NFS score , FIB-4 score, and AST/ALT ratio for the diagnosis of severe fibrosis from the lower stages*

Discussion

Over the past two eras, there have been remarkable improvements in the evaluation of the degree of liver fibrosis with NITs for the diagnosis and to risk stratify patients suffering from fatty liver disease. Their routine usage in medical field as a screening tool for fibrosis and establishing prognosis has truly transformed the clinical practice of hepatologists.[11]

Study done by Polizos et al suggested that APRI could discriminate simple steatosis from steatohepatitis and the combined application of APRI and ELF test can distinguish F0/F1 from F2/F3 fibrosis stages in fatty liver disease.[12]

The substantial result of the current study demonstrate that the FIB-4 score when compared with the APRI index, which is considered the most reliable in the absence of liver biopsy[13], showed a significant correlation. Furthermore, a noteworthy correlation was observed between the NFS and the FIB-4 score. This finding implies that both tests can be utilized in a clinical setting to identify individuals with NAFLD who do not have advanced fibrosis. Using simple and non-invasive tests to rule out advanced fibrosis makes a diagnosis less costly and convenient for patients. With the current influx of NAFLD patients seeking evaluation at GI clinics, the utilization of these non-invasive tests has the potential to considerably decrease the need for other scans such as fibro scan. This would bring about substantial advantages for patients by focusing expensive tests on individuals with a higher likelihood of advanced liver disease, while also generating cost savings.

In a similar study conducted by McPherson et al.[8] researchers found that the FIB-4 had the best AUC (0.86), implying that, based on their study's parameters, the FIB-4 exhibited the highest diagnostic accuracy, with the NFS ranking second in terms of diagnostic performance, with an AUC of 0.81. In our study the AUC for the FIB-4 test was 0.904, which is similar to McPherson. Similar conclusions were also obtained by Drolz et al[14] (FIB-4 AUROC 0.904), and multiple studies reviewed by Lee et al[15] and Hussain et al[16]. A study done on Portuguese population by Rigor, Joana et al reported the AUROC and the NPV of 0.80 and 89.9% for APRI, 0.86 and 93.0% for NFS and 0.88 and 95.7% for FIB-4 respectively.[17]

In addition, all the previous studies cited also arrived at the conclusion that in contrast, the NFS only moderately correlated with the APRI index. In concurrence with those findings, our AUROC value for NFS score was 0.740. There is however some contradictory data available, which in its cohort of 78 young patients with biopsy proven NAFLD showed that when comparing the AUC for ROC curves, all scores including FIB-4 and NFS failed to predict any fibrosis, significant or otherwise. It should be noted however, that this study was contained to young adults, with a limited sample size. The authors have also suggested that stratifying score cutoffs on the basis of age ranges may help eliminate this discrepancy, as these scores have proven their usefulness when applied to middle aged adults such as those on McPherson et al.[8]

A recent study done by Mikolasevic et al comparing

the gold standard liver biopsy with noninvasive tools demonstrated that only the cell death biomarkers and APRI showed moderate accuracy (AUC > 0.7), while NFS, BARD and FIB-4 scores provided poor performance (AUC < 0.70) for advanced fibrosis. Though, a grouping of cell death biomarkers together with the FIB-4 and NFS had demonstrated moderate accuracy, with an AUC of >0.70 for detection of advanced (≥ F3) fibrosis.[18]

Regarding the AST/ALT ratio, 58.7% patients had intermediate fibrosis, whereas 41.3% patients had advanced fibrosis. This is a higher percentage when contrasted with studies such as McPherson et al[8] (19% advanced fibrosis) and Drolz et al[14] (42% moderate fibrosis and 16% advanced fibrosis). This discrepancy may be explained as variability within various populations. More studies, with larger sample sizes may be able to provide more reliable, standardized numbers.

The demographics of study line up well when compared to the broader literature present, and our sample size of 322 patients was at par with or larger when compared to similar studies.[19-20] Additionally we had variability with regards to the severity of fibrosis, be it moderate or severe, when compared to studies done in Pakistan[20,21] as well as those conducted across Europe and North America.[22-25]

Using simple scoring systems, such as the one we utilized, offers a significant benefit because they can be easily derived from clinical and laboratory data. While it is true that calculating the scores may be complex, the individual indices can be effortlessly inputted into a calculator or spreadsheet with a pre-existing formula, making it accessible to all physicians. However, in our study, there is a drawback when considering the various scores or percentage values associated with moderate and severe fibrosis change. Ideally, all participants would have undergone a biopsy for a definitive diagnosis. Nevertheless, in our specific situation, this approach would have been expensive and burdensome for patients, potentially reducing the sample size and the reliability of our study.

The current study has few limitations of having a single center study and use of small sample size for analysis. Also the assessment of severity of liver fibrosis was done using various noninvasive markers and comparison of efficacy was done among each other without the use of gold standard liver histology or elastography.

This study recommends that the approach of employing simple and non-invasive tests can lead to a more cost-effective and convenient approach to evaluate fibrosis among patients suffering from NAFLD. Given the increasing number of fatty liver disease patients, incorporating these tests into clinical practice could substantially reduce the need for more expensive procedures like fibro scan and elastography. Such a shift would prove especially beneficial in settings with limited resources, including countries like Pakistan, where a stronger healthcare framework and early detection are imperative. Moreover, this shift would empower doctors to initiate timely interventions.

Conclusion

This study establishes a strong robust correlation between the APRI index and the FIB-4 score, as well as between the FIB-4 score and the NFS score. These findings highlight the potential medical implication of both the NFS and the FIB-4 score in stratifying NAFLD population with advanced fibrosis which will effectively contribute in the proper management of these patients.

Ethical Approval: The Institutional Review Board, Dow University of Health Sciences, Karachi approved the study vide letter No.Ref: IRB-1842/DUHS/ Approval/ 2021

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Authors' Contribution:

SSS: Conception & design, analysis & interpretation of data, drafting of article, critical revision for important intellectual content, final approval

SN: Conception & design, data collection

SJR: Conception & design, data collection

SZ: Analysis & interpretation of data,

MS: drafting of article

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