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Cross sectional study of comparison of aspartate aminotransferase to platelet ratio index and fibrosis-4 scores in predicting the degree of fibrosis in NAFLD patients in a tertiary care centre.

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Abstract

Background: Non-alcoholic fatty liver disease (NAFLD), a multifaceted disease, includes a wide spectrum of liver damage, currently the most common chronic liver disease. Affecting about 25-30% of adults around the world. The non-invasive assessment scores are useful to assess liver fibrosis where liver biopsy is not accessible. ***Aim:*** To assess the efficacy and performance of the fibrosis index based on two factors FIB-4 and APRI to predict the degree of fibrosis in NAFLD patients. ***Materials and methods:*** The present study was conducted in Department of General Medicine, Akash Institute of Medical Sciences and Research Centre (AIMSRC), Bengaluru, Karnataka, India. This study was initiated after obtaining the Institutional Ethics Committee and informed consent from all the study subjects. This cross-sectional study involved 110 subjects. Patients with NAFLD diagnosed through ultrasound subjects were selected for the study after considering the inclusion and exclusion criteria. Simple random sampling method was followed for recruiting the study subjects. The BMI and WHR were calculated. Demographic data, blood pressure, anthropometrics, clinical details and family history were recorded. Ultrasound of abdomen and pelvis was done. Five ml fasting venous blood samples were collected from all the study subjects, transferred 2 ml into EDTA tube and 3 ml in plain tube and centrifuged. The serum sample was used for analysis of biochemical parameters such as total cholesterol, triglycerides, HDLC were estimated by using Biochemistry fully auto analyzer. LDLC and VLDLC were calculated by Friedlwald's formula. Serum total & direct bilirubin, AST, ALT, ALP were estimated by using Biochemistry fully auto analyzer. EDTA samples were used for complete blood count (CBC) analysis. The APRI and FIB-4 scores were calculated by using the formulas. ***Results:*** In the present study, mean age of the study subjects 46.1±13.9 years, BMI 24.9±1.0 kg/m2, WHR 0.86±0.06, platelets 300.0±76.0, Total Bilirubin 0.78±0.45 mg/dl, Direct Bilirubin 0.33±0.30 mg/dl, AST 64.8±28.4 IU/L, ALT 57.7±22.2 IU/L, ALP 96.7±30.9, total cholesterol 189.0±29.8 mg/dl, Triglycerides 189.0±29.8 mg/dl, HDLC 39.2±9.1 mg/dl, LDLC 103.4±40.1 mg/dl, APRI 0.61±0.44, and FIB-4 value 1.52±0.9.1. In this study, APRI was significantly negatively correlated with platelets (r=-0.544) and positively correlated with AST (r=0.791), ALT (r=0.249) and FIB-4 (r=0.613) whereas FIB-4 showed negative correlation with platelets (r=-0.429), ALT (r=-0.212) and APRI (r=-0.117) and positively correlated with age (r=0.612), AST (r=0.458), TGL (r=.079) and HDLC (r=0.626). ***Conclusion:*** The present study may conclude that APRI and FIB-4 may serve as non-invasive tool for fibrosis in patients with NAFLD.

Keywords: APRI, FIB-4, NAFLD.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD), a multifaceted disease, includes a wide spectrum of liver damage, ranging from the accumulation of fat in >5% of hepatocytes (NAFLD) to non-alcoholic steatohepatitis (NASH), characterized by tissue necroinflammation and possible fibrosis at different stages associated with steatosis. 1 NAFLD has undergone a dramatic epidemiological transition, currently the most common chronic liver disease globally, affecting about 25–30% of adults around the world.2

Its pathological spectrum ranges from simple hepatic steatosis to non-alcoholic steatohepatitis (NASH), fibrosis, cirrhosis, and hepatocellular carcinoma (HCC). 3 Recent evidence indicates that NAFLD accounts for approximately 2.4% of global mortality from liver diseases, surpassing both viral hepatitis and alcoholic liver disease in many developed nations. 4

The pathophysiological intersection between NAFLD and aging presents unique clinical challenges due to age-related alterations in hepatic lipid metabolism, characterized by diminished β-oxidation capacity, mitochondrial dysfunction, and increased pro-inflammatory cytokine production with aging. 5,6

Incidence of NAFLD and its related consequences such as cirrhosis is increasing day by day to a large extent, prognostically the condition is of utmost importance. Presence of fibrosis and degree of fibrosis are considered important factors for the prognosis of NAFLD and in predicting the risk of developing cirrhosis and finally hepatocellular carcinoma. 7

To date, the gold standard of diagnosis remains percutaneous liver biopsy. 8 However, the biopsy is not only an expensive procedure, also an invasive with high risk of complications and sampling errors. Therefore, there is a necessity to investigate new non-invasive biomarkers for early diagnosis of fibrosis. 9 Elastography techniques are a range of methods to that non-invasively assess liver stiffness through the measurement of the velocity of propagation of a shear wave generated by a probe. However, these techniques are limited by the presence of obesity, severe congestion and inflammation. 10

There are some non-invasive scores to diagnose the stage of NAFLD such as FIB-4 (fibrosis-4) and APRI (aspartate aminotransferase/platelet index) and NAFLD fibrosis score (NFS). 11, 12 The non-invasive assessment scores are useful to assess liver fibrosis where liver biopsy is not accessible. In support of this a study done by Mosca A, et al., conducted a study to evaluate the usefulness of four fibrosis scores APRI, FIB-4, NAFLD Fibrosis Score [NFS], and Hepamet in predicting different degrees of fibrosis among children with biopsy-proven NAFLD. They reported that, Hepamet and APRI perform better than NFS and FIB-4 for identifying fibrosis in patients with NAFLD. 13

Another study by Xiao G et al., reported that APRI and FIB-4 correlate with liver fibrosis. However, these two models have low accuracy for predicting HBV-related liver fibrosis in HCC patients. 14 In a study by Xiao G et al., conducted a systemic review and meta-analysis, to systematically review the performance of APRI and FIB-4 in hepatitis B virus (HBV) infection in adult patients and compare their advantages and disadvantages. They suggested that APRI and FIB-4 can identify hepatitis B-related fibrosis with a moderate sensitivity and accuracy. 15 However, there are limited studies were available. Therefore, the present study was aimed to assess the efficacy and performance of the fibrosis index based on 2 factors FIB-4 and APRI to predict the degree of fibrosis in NAFLD patients.

MATERIALS AND METHODS

The present study was conducted in Department of General Medicine, Akash Institute of Medical Sciences and Research Centre (AIMSRC), Bengaluru, Karnataka, India. This study was initiated after obtaining the Institutional Ethics Committee (AIMSRC/IEC/97/2024-25) and informed consent from all the study subjects. Patients with NAFLD diagnosed through ultrasound subjects were selected for the study after considering the inclusion and exclusion criteria. Patient's history was taken, physical examination was done.

Sample Size Calculation

$n=((Z_{(1-\alpha/2)})^2 \cdot P \cdot Q)/d^2$

Where:

- $Z_{(1-\alpha/2)} = 1.96$ at 95% confidence interval
- P is the prevalence or proportion of APRI = 77.8%
- Q = 100 − P = 100 − 77.8 = 22.2%
- d = 7.78 at 10% of prevalence or proportion

Substituting the values:

$n=((1.96)^2 \cdot 77.8 \cdot 22.2)/(7.78)^2$

n = 109.6.

The obtained sample size is 110. Simple random sampling method was followed for recruiting the study subjects.

Inclusion Criteria

Subjects willing to participate in the study, patients aged more than 18 years, non-alcoholic patient, patients with diabetes without comorbidities were included in the study.

Exclusion Criteria:
Subjects not willing to participate in the study, patients with hepatitis infections, patients with other conditions altering the liver function test, patients taking hepato-toxic drugs, patients with hemolytic conditions, patients with hypothyroidism, celiac disease, history of myocardial infarction, dermatomyositis were excluded from the study.

Anthropometric measurements
The BMI was calculated by dividing an individual's weight in kilograms by the square of height in meters. Waist to hip ratio (WHR) was calculated. Demographic data, blood pressure, anthropometrics, clinical details and family history were recorded.

Estimation of biochemical parameters
Five ml fasting venous blood samples were collected from all the study subjects, transferred 2 ml into EDTA tube and 3 ml in plain tube and centrifuged. The serum sample was used for analysis of biochemical parameters such as total cholesterol (cholesterol oxidase/peroxidase), triglycerides (glycerol phosphate oxidase/peroxidase), HDLC (HDLC-Direct) were estimated by using Biochemistry fully auto analyzer. LDLC and VLDLC were calculated by Frieldwald's formula. Serum total & direct bilirubin (Diazo), AST (IFCC), ALT (IFCC), ALP (PNP-AMP). EDTA samples were used for complete blood count (CBC) analysis.

Calculation of Non-invasive Fibrosis Scores:
In this study, we calculated the APRI and FIB-4 scores.
The APRI scores
APRI=((AST Level (U/L))/(AST (Upper Limit of Normal) (IU/L)))/(Platelet Count(〖10〗^9/L))×100

FIB-4 scores were calculated by using the formula:
FIB-4=(Age (years)×AST Level (U/L))/(Platelet Count(〖10〗^9/L)×√(ALT Level (U/L)))

Statistical Analysis
Results were expressed as mean±SD. Spearman's rho correlation was applied to correlate APRI and FIB-4 with study parameters. The p value ($p < 0.05$) was considered as statistically significant. Data analysis was done by using SPSS 22.0.

RESULTS

In the present study, mean age of the study subjects 46.1±13.9 years, BMI 24.9±1.0 kg/m^2, WHR 0.86±0.06, platelets 300.0±76.0, Total Bilirubin 0.78±0.45 mg/dl, Direct Bilirubin 0.33±0.30 mg/dl, AST 64.8±28.4 IU/L, ALT 57.7±22.2 IU/L, ALP 96.7±30.9, total cholesterol 189.0±29.8 mg/dl, Triglycerides 189.0±29.8 mg/dl, HDLC 39.2±9.1 mg/dl, LDLC 103.4±40.1 mg/dl, APRI 0.61±0.44, and FIB-4 value 1.52±0.9.1 as shown in table 1.

Table 1. Demographic biochemical parameters of study subjects.

Parameters	Cases, (n=110) Mean±SD
Age (Years)	46.1±13.9
BMI (kg/m^2)	24.9±1.0
WHR	0.86±0.06
Platelets, 10^9/L	300.0±76.0
Total Bilirubin (mg/dl)	0.78±0.45
Direct Bilirubin (mg/dl)	0.33±0.30
Aspartate transaminase (AST) (IU/L)	64.8±28.4
Alanine transaminase (ALT) (IU/L)	57.7±22.2
Alkaline phosphatase (ALP) (IU/L)	96.7±30.9
Total Cholesterol (mg/dl)	189.0±29.8
Triglycerides (mg/dl)	179.1±51.2
HDLC (mg/dl)	39.2±9.1
LDLC (mg/dl)	103.4±40.1
APRI	0.61±0.44
FIB-4	1.52±0.9.1

In this study, APRI was significantly negatively correlated with platelets (r=-0.544) and positively correlated with AST (r=0.791), ALT (r=0.249) and FIB-4 (r=0.613) whereas FIB-4 showed negative correlation with platelets (r=-0.429), ALT

(r=-0.212) and APRI (r=-0.117) and positively correlated with age (r=0.612), AST (r=0.458), TGL (r=.079) and HDLC (r=0.626) as shown in table 2.

Table 2: Correlation of APRI and FIB-4 with study parameters.

Parameters	APRI		FIB-4	
	r-value	p-value	r-value	p-value
Age (Years)	0.112	0.245	0.612**	0.000
BMI (kg/m^2)	-0.053	0.582	-0.020	0.836
WHR	-0.123	0.200	-0.093	0.331
Platelets	-0.544**	0.000	-0.429**	0.000
Total Bilirubin (mg/dl)	0.215*	0.024	0.155	0.106
Direct Bilirubin (mg/dl)	0.109	0.256	0.028	0.774
Aspartate transaminase (AST) (IU/L)	0.791**	0.000	0.458**	0.000
Alanine transaminase (ALT) (IU/L)	0.249*	0.021	-0.212*	0.026
Alkaline phosphatase (ALP) (IU/L)	0.055	0.572	-0.014	0.886
Total Cholesterol (mg/dl)	-0.058	0.550	-0.170	0.075
Triglycerides (mg/dl)	-0.117	0.223	.079**	0.036
HDLC (mg/dl)	-0.067	0.484	0.626**	0.000
LDLC (mg/dl)	-0.071	0.464	-0.183	0.056
FIB-4	0.613**	0.000	-	-
APRI	-	-	-0.117**	0.000

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

Table 3 shows the staging of NAFLD based on APRI and FIB-4 score.

Table 3: Staging NAFLD based on APRI and FIB-4 scores

Stage	APRI (n, %)	FIB-4 (n, %)
Mild	43 (39.0%)	58 (52.7%)
Moderate	62 (56.3%)	42 (38.1%)
Severe	5 (4.5%)	10 (9.0%)
Total	**110 (100%)**	**110 (99.8%)***

DISCUSSION

The prevalence of NAFLD is increasing globally. The progression and stage of liver fibrosis determine the prognosis and treatment of the disease. For clinical decision-making and follow-up, accurate quantification of liver fibrosis is of paramount importance. Only a liver histological examination can accurately confirm the existence of concomitant liver fibrosis, necroinflammatory activity, and steatosis. Liver biopsy is the gold standard test for evaluating liver fibrosis stages and due to the invasive nature of the procedure, the small size of the specimen, sampling risks, and inter-observer variability in the histopathological examination can restrict the routine use of liver biopsy. 16 Due to the non-invasive nature of transient elastography, several recommendations also state that it is an excellent way to assess liver fibrosis. 17

In the present, APRI was significantly negatively correlated with platelets (r=-0.544) and positively correlated with AST (r=0.791), ALT (r=0.249) and FIB-4 (r=0.613) whereas FIB-4 showed negative correlation with platelets (r=-0.429), ALT (r=-0.212) and APRI (r=-0.117) and positively correlated with age (r=0.612), AST (r=0.458), TGL (r=.079) and HDLC (r=0.626).

In support of our study, a cross-sectional study conducted by Amernia B et al., including patients with NAFLD to compare fibrosis-4 (FIB-4), aspartate aminotransferase (AST) to platelet ratio index (APRI), and aspartate aminotransferase/alanine aminotransferase (AST/ALT) ratio with FibroScan for the assessment of hepatic fibrosis in patients with NAFLD. They reported that APRI appears to be the most appropriate substitute of FibroScan for the detection of significant fibrosis in NAFLD patients. FIB-4 was the second best, suggesting that in case of FibroScan unavailability, APRI and FIB-4 are the best indices to assess liver fibrosis in NAFLD patients. 18 Similarly, another cross-sectional study conducted by Moosavy SH et al., included patients with Chronic hepatitis B (CHB) reported that, APRI can rule out 95.4% of F3/F4 of liver fibrosis and rule in any grade of liver fibrosis in CHB patients by 90.78%. Therefore, APRI appears to be the best substitute for

FibroScan in the assessment of liver fibrosis in patients with CHB. 19 In a comparative cross-sectional study by Ikram H et al., to compare the extent of liver fibrosis using fibroscan and subsequently compare it with AST/ALT ratio and AST to platelet ratio index (APRI). They reported that Early fibro scan testing and serum markers can help in early diagnosis and timely initiation of treatment for NAFLD. 20

Alam M.S et al., conducted a cross-sectional study to evaluate the diagnostic performance of non-invasive hepatic indices (FIB-4, APRI, and AST/ALT ratio) compared with FibroScan for assessing the stage of fibrosis in individuals with NAFLD and T2DM. They reported that FIB-4 was the most reliable non-invasive tool for detecting advanced fibrosis in patients with NAFLD and T2DM. 21.

CONCLUSION

The present study may conclude that, APRI was significantly negatively correlated with platelets and positively correlated with AST, ALT and FIB-4, whereas FIB-4 showed negative correlation with platelets, ALT, APRI and positively correlated with age, AST, TGL and HDLC. Therefore, APRI and FIB-4 may serve as non-invasive tool for fibrosis in patients with NAFLD. Further studies with large sample size are recommended.

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