PERFORMANCE OF NON-INVASIVE BIOCHEMICAL MARKERS FIB-4 AND APRI SCORE FOR ASSESSMENT OF LIVER FIBROSIS IN PATIENTS OF NON-ALCOHOLIC FATTY LIVER DISEASE

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ABSTRACT

Background & objectives: NAFLD progresses from simple steatosis to non alcoholic steatohepatitis, cirrhosis & hepatocellular carcinoma. But the spectrum is not linear, all steatosis patients may not develop NASH & cirrhosis but may also regress in a backward direction on early diagnosis and appropriate treatment. Two hit hypothesis is proposed for pathogenesis, where mitochondrial dysfunction with oxidative stress and activation of stellate cells leads myofibroblast to secrete collagen. Liver biopsy is the gold standard procedure for diagnosis. But due to limitations and associated risks non-invasive biomarkers are being developed, which are low cost and have easy accessibility. Some indirect markers of liver fibrosis are ratio of AST/ALT, APRI score, FIB-4 and fibroscan. Fibroscan uses to measure tissue stiffness. On this background the study aims at correlating non-invasive markers FIB-4 and APRI score with degree of fibrosis detected by Fibroscan.

Materials & Methods: Hundred diagnosed cases of NAFLD and age and sex matched hundred healthy controls were chosen for the study. BMI, FBS, lipid profile, liver function test, AST/ALT ratio, APRI score and fibroscan were measured in both groups.

Results: FIB-4 and APRI score were significantly higher (p, 0.001) in NAFLD cases than control group. There was a significant positive correlation between FIB-4 and APRI score with fibroscan.

Conclusion: FIB-4 and APRI score may be considered as a cost effective marker for fibrosis in NAFLD.

Key Word: NASH, NAFLD, FIB-4 APRI, Fibroscan

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) has emerged as an epidemic and has become the most prevalent liver disease affecting around two billion people in the world .[1,2]The situation is alarming in India too, with a prevalence rate of NAFLD ranging between 14.6-42% [3]. The global incidence of NAFLD has been projected to reach 56% in the next decade [4].

NAFLD encompasses a spectrum of diseases, ranging from simple steatosis to Non Alcoholic Steatohepatitis (NASH) characterized by hepatocyte death, inflammation and the development of fibrotic lesions. This may progress further to cirrhosis and eventually to hepatocellular carcinoma (HCC) and/or end-stage liver disease (ESLD).[2]Advanced cases of NAFLD can progress to HCC even in absence of cirrhosis [4]. Either of the two conditions (HCC and ESLD) severely affects life expectancy, the only treatment being liver transplantation.

NAFLD is also associated with an increased risk of other metabolic disorders, including insulin resistance, type 2 diabetes mellitus (T2DM), dyslipidaemia and hypertension.[5, 6]

The initial stages of NAFLD are reversible and, not all patients of simple steatosis develop NASH & cirrhosis, Patients of NASH can also revert to Simple Steatosis and to normal on early diagnosis and proper treatment.[4]Various invasive & non- invasive procedures are developed to diagnose fibrosis in liver.

Liver biopsy is considered the gold standard to diagnose fibrosis in NAFLD patients but it has lots of limitations. Monitoring of NAFLD patients who are mostly asymptomatic , by repeated

biopsy for disease progression and response to treatment is not advisable.[7] So there is a need to investigate non- invasive procedures for diagnosis of fibrosis in NAFLD patients.

Special blood tests or a combination of tests have been used to evaluate possible liver fibrosis. One such indirect bio markers is aspartate transaminase to platelet Ratio Index (APRI) which uses AST and platelet count. Fibrosis-4 (FIB-4) is another scoring system to grade liver fibrosis using a combination of patient's age, platelet count, aspartate transaminase (AST) and alanine transaminase (ALT), all easily available to a primary care physician, besides being inexpensive[8].

Fibroscan is a bedside test with immediate and reliable results with high patient acceptance. A shear wave is produced in liver by transmitting vibrations of mild amplitude and low frequency. The tissue stiffness correlates with the speed of the wave. Obesity is a possible limitation to its efficacy.[9]It has limited availability and due to its high cost and need of skilled hands restricts its frequent use for the follow-up of general population in developing countries like India.[10]

The present study is taken up to correlate non-invasive tests FIB 4 and APRI SCORE with degree of fibrosis diagnosed by fibroscan in NAFLD patients

AIM OF THE STUDY

The aim of this study is to compare the non-invasive biochemical scores FIB-4 and APRI score with the degree of fibrosis diagnosed by fibroscan in NAFLD patients.

MATERIAL AND METHOD

This study was conducted in the department of biochemistry in collaboration with department of hepatology at S.C.B. medical college and hospital Cuttack from October 2016 to September 2017.The study was approved by institutional ethical committee & informed consent was obtained.

100 diagnosed patients of NAFLD within the age group 20-60 years, attending OPD of department of hepatology were included in this study. Equal number of age and sex matched healthy volunteers were included as controls.

Subjects with alcohol habit >20 gm/day, diabetes mellitus, impaired biliary excretion, impaired renal function, recent hepatic infection and patients taking drugs modifying liver function were excluded from the study.

The biochemical parameters, fasting blood sugar, liver function test, lipid profile, urea and creatinine were estimated by autoanalyser TOSHIBA 120 FR using commercial kits and platelet count was done by hydrodynamic focusing method. Height and weight of all cases and control was taken.

FIB-4 was determined by the following formula[8]:

Age (years) × AST (IU/L) / {platelet count (10^9/L) × ALT (IU/L) $^{1/2}$}

APRI score was calculated using the formula: [22]

[(AST/upper limit of the normal AST range) X 100]/Platelet Count.

STATISTICS

All results were expressed as mean ± SD. Test of significance was done by unpaired student't' test. 'P' value <0.05 was taken as significant. Pearson coefficient of correlation was used for correlation. All statistically analysis was done by SPSS version 24 software.

RESULTS

The age and sex distribution of study population is shown in table-1. The study was conducted among people of 20-60 years of age. The mean age of control was 41.27±11.07and cases was 42.46± 10.59 which were comparable. The ratio between male and female was 59:41and 66:34

in control and case. The BMI was statistically significantly ($p<0.001$) higher in cases (28.31±3.67) than control (24.18±3.16).

Table-1: Age and Sex distribution with BMI in study population

	Control(n=100)	**Cases(n=100)**
Age	41.27±11.07	42.46±10.59
Males	59%	66%
Females	41%	34%
BMI(kg/m2)	24.18±3.16	28.31±3.67

The values of Fasting Blood Sugar (FBS), urea, creatinine, cholesterol, LDL, VLDL, Direct Bilirubin, Total bilirubin show no statistically significant difference between control and cases. Values of triglyceride, VLDL, AST, ALT, and Alkaline Phosphatase show a statistically significant rise in cases ($p<0.001$) than control group. While HDL shows a statistically significant decrease in cases ($p<0.05$) than control.

Table-2: Comparison of biochemical parameters (FBS, lipid profile & LFT) in study population

Parameter	**Control (n=100)**	**Cases (n=100)**	**'p' value**
FBS (mg%)	110.40 ± 9.08	106.40 ± 14.6	NS
Urea (mg%)	22.52 ± 9.54	20.54 ± 6,42	NS
Creatinine (mg%)	0.63 ± o.20	0.85 ± 0.26	NS
Cholesterol (mg%)	175.98 ± 16.47	191.28 ±43.41	NS
TG (mg%)	137.22 ± 36.22	236.08 ± 80.7	0.001
HDL (mg%)	46.08 ± 8.04	37.62 ± 9.14	0.05
LDL (mg%)	103.50 ± 16.64	106.26 ± 39.93	NS
VLDL (mg%)	27.90 ± 7.72	47.64 ± 19.93	0.001
D.Bil (mg%)	0.40 ± 0.09	0.42 ± 0.11	NS
T. Bil (mg%)	0.96 ± 0.16	0.94 ±0.15	NS

AST (IU/L)	41.52 ± 18.07	65.44 ± 51.82	0.001
ALT (IU/L)	36.54 ± 18.23	55.16 ± 42.46	0.001
Alk.P (IU/L)	205.12 ± 85.20	266.12 ± 11.25	0.001

There is no significant difference in platelet count and AST/ALT ratio between cases and control groups. There is a statistically significant increase in fibroscan score, FIB 4 and APRI score in cases and control group ($p<0.001$).

Table-3: Comparison of special parameters among the study population

	Control (n=50)	**Cases (n=50)**	**'p' value**
Total platelet count (no/ml)	3.1± 0.50	2.62± 0.65	NS
Fibroscan (kPa)	4.06± 0.90	15.00 ± 7.5	0.001
FIB 4	0.77±0.31	1.8±0.52	0.001
AST/ALT	1.39 ± 0.70	1.35± 0.78	NS
APRI	0.33± 0.16	0.61± 0.46	0.001

Both FIB 4 and APRI showed a positive correlation with fibroscan score. There is a statistically significant.($p<0.02$) positive correlation between FIB-4 with fibroscan. APRI score also shows a positive correlation with fibroscan.

Table-4: Correlation of Fibroscan with FIB 4 and APRI score

Fibroscan	**'r' value**	**'p' value**
FIB 4	0.312	0.02
APRI score	0.78	0.04

DISCUSSION

The fat content of liver mostly triacylglycerol is <5% of weight. When this increases to > 5-10% of weight in absence of excessive alcohol consumption, the term NAFLD is appropriate.[11]

NAFLD encompasses a spectrum of disease severity ranging from simple steatosis to more advanced disease characterized by hepatocyte death, inflammation and the development of fibrotic lesions (NASH).

"Two hit theory" suggests that in first hit, there occurs steatosis & injury followed by "second hit" leading to inflammation and fibrosis. [12, 13] Activation of stellate cells causes fibrogenic and contractile myofibroblasts to secrete large amount of collagen.[14]It is estimated that NASH is present in 60% of patients with NAFLD who undergo a liver biopsy and 41% of patients with NASH exhibit considerable fibrosis1. Therefore, early diagnosis and treatment is necessary to avoid complication.

Various invasive & non-invasive procedures are adopted to diagnose fibrosis. Liver biopsy, though gold standard, is an invasive procedure and has many limitations.[15,16] Non invasive serum markers which are cost effective, easily available and patient complaint are being investigated.

In our study it was observed that BMI of cases was significantly raised in comparison to control group ($p<0.001$) (table -1) which is in agreement with the study by Marchesini et al.[17] Increase flux of fatty acids due to visceral adipocity is the cause of NAFLD. In the Dionyoss Nutrition and liver study, Bedogni et al found BMI is an independent marker of NAFLD.[18]

We found that there was statistical increase in TG and VLDL levels when compared to control group ($p<0.001$). HDL showed a statistically significant low value in cases ($p<0.05$) which is in conformation with study by Marchesini et al[17]and Ryan et al.[19]

In the present study, there was significant higher value of AST, ALT and Alkaline phosphatase ($p<0.001$) in cases compared to control group. The rise of liver enzymes is due to inflammation and injury to liver cells and this injury along with upgrading fibrosis leads to the progressive rise in liver enzymes This observation is in agreement with the findings of Berasian et al.[20]

There was no statistical difference in platelet count & AST/ALT ratio between cases & control group. Fibroscan score showed a significant higher value in cases ($p<0.001$) compared to control group. Wong et al has demonstrated usefulness of fibroscan in cases of both whites & Asian origin.[21]

Our study showed a significant higher APRI score in cases than control group ($p<0.001$). Lorez-a-del-Castillo et al stated that APRI was capable of predicting significant fibrosis in NAFLD.[22]FIB-4 was also significantly high in case as compared to controls which is in accordance with previous studies. (19) Both APRI score and FIB-4 showed a positive correlation with Fibroscan.

Fibroscan though very accurate is available only at higher centres, requires skilled hands and is very costly. Mild/moderate NAFLD patients are mostly asymptomatic. Use of liver biopsy which is accompanied by pain, risk of bleeding and injury to internal organs is not justified in these patients.[9]

So biochemical scores can be used to detect early fibrosis in NAFLD patients and their regular follow up with better patient compliance even at the primary health care level. They will also reduce the number of referrals of NAFLD patients to higher centres.[22]

CONCLUSION

Based on this study it is concluded that both FIB-4 and APRI scores can be used to detect fibrosis in NAFLD patients. This is the need of the hour in our country where resources are limited. Fibroscan though very accurate is limited and needs skilled hands and liver biopsy which is considered the gold standard has many limitations. With the help of these scores, NAFLD patients can be monitored regularly for disease progression even at the primary health care level. Early diagnosis and regular follow up will certainly reduce complications which imposes heavy economic burden to the families as well as the country.

REFERENCE

1. Lazarus JV, Colombo M, Cortez-Pinto H, et al.: NAFLD - sounding the alarm on a silent epidemic. Nat Rev Gastroenterol Hepatol. 2020, 17:377-379. 10.1038/s41575-020-0315-72.
2. Sivell C: Non alcoholic fatty liver disease: a silent epidemic. Gastroenterol Nurs. 2019, 42:428-434.10.1097/SGA.0000000000000443.
3. Mishra D, Dash KR, Khatua C, et al.: A study on the temporal trends in the etiology of cirrhosis of liver in coastal eastern Odisha. Euroasian J Hepatogastroenterol. 2020, 10:1-6. 10.5005/jp-journals-10018-1312.
4. Huang DQ, El-Serag HB, Loomba R: Global epidemiology of NAFLD-related HCC: trends, predictions, risk factors and prevention. Nat Rev Gastroenterol Hepatol. 2021, 18:223-238. 10.1038/s41575-020-00381-6.
5. Masarone M, Rosato V, Dallio M, et al.: Role of oxidative stress in pathophysiology of non alcoholic fatty liver disease. Oxid Med Cell Longev. 2018, 2018:9547613. 10.1155/2018/9547613.
6. Kasper P, Martin A, Lang S, Kütting F, Goeser T, Demir M, Steffen HM: NAFLD and cardiovascular diseases: a clinical review. Clin Res Cardiol. 2021, 110:921-937. 10.1007/s00392-020-01709-7.
7. Malekzadeh R, Poustchi H: Fibroscan for assessing liver fibrosis: an acceptable alternative for liver biopsy: Fibroscan: an acceptable alternative for liver biopsy. Hepat Mon. 2011, 11:157-158.
8. Shiha G, Seif S, Eldesoky A, et al.: A simple bedside blood test (Fibrofast; FIB-5) is superior to FIB-4 index for the differentiation between non-significant and significant fibrosis in patients with chronic hepatitis C. Hepatol Int. 2017, 11:286-291. 10.1007/s12072-017-9796-z.

9. Fallatah HI, Akbar HO, Fallatah AM: Fibroscan compared to FIB-4, APRI, and AST/ALT ratio for assessmentof liver fibrosis in Saudi patients with nonalcoholic fatty liver disease. Hepat Mon. 2016,:e38346.10.5812/hepatmon.38346.
10. Cox BD, Trasolini R, Galts C, Yoshida EM, Marquez V: A188 comparing the performance of fibrosis-4 (FIB-4) and non-alcoholic fatty liver disease fibrosis score (NFS) with fibroscan scores in non-alcoholic fatty liver disease. J Can Assoc Gastroenterol. 2020, 3:59-60. 10.1093/jcag/gwz047.187.
11. Cairns SR, Peters TJ. Biochemical analysis of hepatic lipid in alcoholic and control subjects. Clin Sci 1983; 65:645-52.
12. Day CP, James OF. Steatosis: a tale of two hits? Gastroenterology 1998; 114:842-5
13. Day CP. From fat to inflammation. Gastroenterology 2006; 130:207-10.
14. Schuppan D, Ruchl M, Somasundaram R, Hahn EG. Matrix as a modulator of hepatic fibrogenesis. Semin Liver Disease Aug 2001; 21(3):35-72.
15. Bedosa P, Dargere D, Paradis V. Sampling vaiability of liver fibrosis in chronic hepatitis C. Hepatology 2003;38:1449-1457.
16. Cadranel JF, Rufat P, Degos F. Practices of liver biopsy in France results of a prospective nationwide survey. For the group of Epidemiology of the French association for the study of the liver (AFEF). Hepatology 2000; 32:477-481.
17. Marchesini G, Bugianesi E, Forlani G, et al Non alcoholic fatty liver steatohepatitis and metabolic syndrome, Hepatology 2003;37: 917-929.
18. Bedogni G, Miglioni L, Masutti F, Tiribelli C, Marchesini G, Bellatani S. Prevalence of and risk factors for non alcoholic fatty liver disease: the Dionysos nutrition & liver study. Hepatology 2005; 42:44-52.

19. C Ryan, Ki Chul Sang, Bum Soo Kim, et al. Relationship between estimates of adiposity insulin resistance and alcoholic fatty liver disease in a large group of Nondiabetic Korean Adults. Diabetes Care 2007 Aug; 30(8):2113-2118.
20. Berasain C, Betes M, Panizo A. Pathological & virological finding in patients with persistent hypertransaminasaemia of unknown aetiology. Gut 2000; 47:429-435.
21. Wong GL, Kwok R, Choi KC, Zhang Y, Chan HL, Luk AO, Shu SS, Chan AW, Yeung MW, Chan JC, Kong AP, Wong VW. Screening diabetic patients for non alcoholic fatty liver disease with controlled attenuation parameter and liver stiffness measurements: a prospective cohort study. Gut 2016; 65:1359-68.
22. A Loaeza-del-Castillo, F. Paz-Pineda, E. Oviedo-C'ardenas, F. S'anchez-Avila and F. Vargas-Vor'acko'a," AST to platelet ratio indx (APRI) for the non invasive evaluation of liver fibrosis". Annals of Hepatology 2008; 7(4):350-357.