FIB-4 as a non-invasive marker in predicting fatty liver in select populations from Tamil Nadu State.

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Research Article

Keywords: FIB-4 score, fatty liver, non-alcoholic fatty liver disease

Posted Date: February 28th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1386198/v1>

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1Original article**2FIB-4 as a non-invasive marker in predicting fatty liver in select populations
3from Tamil Nadu State.****4Running title – FIB 4 in predicting fatty liver in select populations**

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21Ethical committee approval done. Ref No CSP-MED/20/DEC/64/211

22Grant Support: None

23Potential conflicts of interest: None

24Acknowledgements: None

25

26**Author contributions:**

27JV made the study concept and design; acquisition of data done by VYB, AT, MK,
28statistical analysis done by JV; drafting of manuscript done by JV, VYB, critical
29revision of manuscript done for important intellectual content done by JV, VYB;
30administrative and technical support by JV.

31

32Manuscript was not presented previously

33

34Manuscript has been read and approved by all the authors and each author believes
35that the manuscript represents honest work.

36

37Details –

38Abstract count -224

39Text word count -1692

40Total pages -12

41Tables -9

42Figures – 2

43

44

45

46

47**Cover Letter**

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49To,

50The Editor

51Hepatology International.

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53Dear Sir/Madam,

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Sub: Submission of Manuscript for publication

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56We intend to publish an article entitled “**FIB-4 as a non-invasive marker for predicting fatty liver in**
57**select population of Tamilians from Tamil Nadu State.**” in your journal as an Original Article.

58On behalf of all the contributors I will act and will correspond with the journal from this point onward.

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61 Prior publication – None

62 Support - None

63 Conflicts of interest - None

64 Permissions - Nil

65

66 Thanking you,

67

Yours respectfully,

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Dr V Y Bhargav.

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75 **Abstract**

76**Background:** FIB 4 score is known to have a significant negative and positive
77predictive value in predicting lower and higher grades of liver fibrosis.

78**Aim:** To determine the cut off of FIB-4 in patients with a normal ultrasound and in
79those with Fatty Liver (all grades) as a predictor of fibrosis.

80**Methods:** Prospective data on hemogram, liver biochemistry and ultrasound
81(USG) were collected in Master Health Check (MHC). USG findings were
82classified as normal Grade 1, 2 and 3 FL.FIB-4 were calculated. Patients with
83known liver disease were excluded.

84**Statistical Analysis:** Appropriate non parametric tests were used. AUROC was
85obtained for FIB-4 cut off. SPSS version 23v was used.

86**Results:** There were 741 subjects (274 men; 64%). 418 (56.4%) were > 45 years.
87Liver was normal in 297 (40.1%); grade 1, 2 and 3 FL in 293 (39.5%), 114 (15.4%)
88and 37 (5%) subjects respectively. The overall mean FIB-4 score was high in males
89(1.37 ± 1.06 vs 1.15 ± 0.83 , p value 0.009) and remained so for age > 45 years
90(1.69 ± 1.09 versus 1.33 ± 0.88 , p value 0.000). Mean FIB-4 scores increased with
91grades of FL (p 0.126). AUROC was 0.538, 0.516 and 0.602 respectively for Gr 1,
922 and 3 respectively.

Conclusion: FIB-4 score in our study is lower than that reported in the west with poor prediction for any grade of FL.

Key words: FIB-4 score, fatty liver, non-alcoholic fatty liver disease

Abbreviations –

US – Ultrasound, FL – Fatty liver – Fatty liver, MHC -Master health check, AST-aspartate transaminase, ALT – alanine transaminase, NAFLD – nonalcoholic fatty liver disease, NASH – nonalcoholic steatohepatitis, NFS – NAFLD fibrosis score, BMI – body mass index, PPV -Positive predictive value, NPV – Negative predictive value, PLR -Positive likelihood ratio, NLR – Negative likelihood ratio, CI – Confidence interval, AUROC – Area under the receiver operator characteristic curve

Introduction

FIB-4 score is a non-invasive tool to measure liver fibrosis. The score was developed to correlate with Ishak stages of fibrosis (by liver biopsy) with 3 stages: 0-2 (mild fibrosis), 3-4 (moderate) and 5-6 (severe fibrosis). The FIB-4 was originally developed to be used in assessing fibrosis in the hepatitis C virus/human immunodeficiency virus co-infected population.¹ The score includes readily available clinical data of: age, AST, ALT and platelet counts. Shah et al² validated the FIB-4 for use in NAFLD, in their study comparing against other non-invasive scoring systems (Fibro scan, BARD, NFS and NASH scores), using a cohort of 541 patients from the NASH CRN network. In their study, the AUROC for FIB-4 in recognizing patients with advanced fibrosis (F3-F4) was 0.802 (95% CI: 0.758–

1180847), which was similar to the NFS but higher than the other scoring systems. A
119FIB-4 score of ≥ 2.67 , had an almost 80% positive predictive value and a value of
120 ≤ 1.30 had a negative predictive value of 90% for advanced fibrosis. These results
121were similar in other studies comparing the performance of non-invasive markers
122of advanced fibrosis in NASH.^{3,4,5,6} FIB-4 also has shown good performance in the
123prediction of mild to moderate fibrosis in NAFLD. Comparing several noninvasive
124models for interpreting and predicting hepatic fibrosis in a NAFLD patient, a FIB-4
125cut-off of 1.43 had the best AUROC (0.821; 95% CI: 0.75–0.891) to detect stage 1
126fibrosis or higher.⁷ Liver biopsy study remains to be the gold standard in assessing
127inflammation and fibrosis for diagnosis and to prognosticate patients with NAFLD
128but remains far from ideal, with generalized issues of safety(invasiveness) and
129sampling error. Recent years have seen the development of various markers that
130are able to accurately assess necroinflammation, fibrosis, as a viable alternative to
131liver biopsy. This is especially important when taken into consideration that serial
132assessments are required over the course of a patient's lifetime to monitor the
133progression of disease, decide on the intervention and track the response to
134treatment. Scoring systems such as NFS (NAFLD fibrosis score), FIB-4 and APRI
135(AST to platelet ratio index) are easily calculated using commonly available
136parameters and are also helpful tools to allow the physician to recognize patients
137with advanced fibrosis. Systems to assess liver stiffness measurement, such as
138Fibro scan®, have gained popularity in recent times in being highly accurate and
139reproducible, to assess the presence of fibrosis and detect advanced liver disease.
140However, a majority of the non-invasive tests are less reliable and not as efficient
141as liver biopsy to differentiate between the early stages of fibrosis (F1 to F2). The
142role, therefore, of liver biopsy study in this current era is to give definitive proof of

143NASH, especially in patients with a concomitant diagnostic dilemma, such as viral
144hepatitis (B&C), autoimmune hepatitis, and drug-induced liver injury, and to allow
145the assessment of early fibrosis when non-invasive methods are unable to provide
146adequate clarity.

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148**Aim:**

149To determine the cut off of FIB-4 in patients with a normal ultrasound and in those
150with Fatty Liver (all grades) as a predictor of fibrosis.

151**Material and methods:**

152We undertook a cross-sectional study in patients undergoing MHC that included an
153ultrasound scan of the abdomen and baseline information such as age, gender,
154BMI, complete hemogram and liver biochemistry. Ultrasound findings were
155categorized as normal and those with Grade 1, 2 and 3 FL respectively using the
156standard criteria. Patients with known chronic liver disease (cirrhosis and no
157cirrhosis) of any etiology and those with space occupying lesions in the liver were
158excluded. Fibro scan data was available for less than 10 per cent of patients and
159hence not considered during analysis. These patients were part of another study
160done from same center.

161**Statistical methods:** IBM SPSS version 22 was used for statistical analysis.

162Fibrosis was considered as primary outcome variable and FIB-4 score was
163considered as primary explanatory variable. Age and gender parameters were
164considered as other explanatory variables. Mean and standard deviation was
165calculated for quantitative variables and frequency and proportion for categorical
166variables. All quantitative variables were checked for the normal distribution

within each category of explanatory variable by using the visual inspection of histograms and normality Q-Q plots. Shapiro-wilk test was also conducted in the study to assess normal distribution. Shapiro-wilk test p value of >0.05 was considered as the normal distribution. For normally distributed quantitative parameters the mean values were compared between the study groups using ANOVA (>2 groups). The utility of Fib-4 score in predicting any fibrosis was assessed by Receiver Operative curve (ROC) analysis. Area under the ROC curve along with its 95% CI and p value were calculated; 0.98 was taken as the cut off value. The sensitivity, specificity, predictive values and diagnostic accuracy of the screening test with the predicted cut off values along with their 95% CI were obtained. p value < 0.05 was considered statistically significant in the study.

Results:

Of the 990 subjects screened, data was available for 741 patients. Table 1-5 shows the demographic characteristics of patients in the study with normal and the 3 grades of FL. 297 (40.1%) patients had a normal US, 293 (39.5%) grade 1 FL, 114 (15.4%) grade 2 FL and 37 (5%) grade 3 FL. There were 474 (63.9%) males and 267 (36.03%) female patients. The overall mean age was 48.21 ± 13.47 years with majority of patients being above 45 years of age (418: 56.4%). Table 6 and Fig 1 shows the comparative mean FIB-4 score and the 5th and the 95th percentile cut off in patients with normal US and the 3 grades of FL. There is an increasing grade of FIB-4 score with increasing grades of FL, significantly more for Gr 3 FL (p value 0.03) which is statistically significant, with near similar mean scores in normal US and patients with grade 1 FL. (p value 0.907)

The mean FIB-4 score was higher in males suggesting advanced fibrosis (1.37 ± 1.06 vs 1.15 ± 0.83 , p value 0.009). Correlating FIB-4 score and its relation with age, the mean FIB-4 score was significantly higher in patients >45 years (1.55 ± 1.02 vs

193 0.97 ± 0.83 , p value 0.000). On comparing males and females <45 years, FIB-4
 194 score was higher in males (1.01 ± 0.90) than females (0.87 ± 0.66). However, this was
 195 not statistically significant. On the other hand, in subjects >45 years of age, FIB4
 196 score was significantly higher in males (1.69 ± 1.09 vs 1.33 ± 0.88 , p 0.000).
 197 Correlating FIB-4 score and its relation to grades of FL on US, the mean FIB4 scores
 198 increased with increase in the grades of FL. However, the differences in the values
 199 were not statistically significant (p 0.126). The area under the receiver operating
 200 curve (AUROC) for grade 1, grade 2 and grade 3 FL was 0.538, 0.516 and 0.602
 201 respectively, suggesting poor prediction between FIB4 values and any grades of FL
 202 of ultrasound.

203 Discussion

204 Most studies on FIB-4 have been in patients with chronic HCV infection^{1,8}. Cut off
 205 values for predicting fibrosis has varied in different series. Vallet – Pichard et al¹,
 206 in their series of 842 patients had a liver biopsy for chronic HCV infection, the FIB-
 207 4 index of >3.25 predicted patients with severe fibrosis (F3-F4) and cirrhosis with
 208 an AUROC of 0.85 (95% CI 0.82-0.89) and 0.91 (95% CI 0.86-0.93) respectively and
 209 a 82.1% of positive predictive value and specificity of 98.2%. A FIB-4 index < 1.45
 210 had a negative predictive value of 94.7% to exclude severe fibrosis with a
 211 sensitivity of 74.3%. Using the outer range of 1.45 and 3.25, the authors could
 212 accurately classify the cases on liver biopsies in 72.8% cases^{1,8}. Correlated
 213 similarly, in a study by Karic U et al⁸ of 142 patients with liver biopsy for staging
 214 chronic HCV infection, found patients with non-severe fibrosis were younger, had
 215 higher platelet counts and lower transaminase levels; FIB4 had an AUROC of
 216 0.875. The FIB-4 score ranged from 0.1 to 3.48 in those with non-severe fibrosis

and 1.09 to 5.66 in patients with severe fibrosis. In this particular study, FIB-4 score could predict severe fibrosis fairly accurately (AUC = 0.875, $p < 0.001$). The cut-off point for the best balance between sensitivity and specificity was established to be 1.39 with a sensitivity of 86.67% and a specificity of 72.32%. The ROC analysis confirmed that FIB-4 score is associated with a sensitivity of 100% (95% CI = 88.4% - 100%) and that the cut-off value was 1.08. None of the patients with severe fibrosis or cirrhosis had a FIB-4 lower than 1.08 (near 100% sensitivity). Shukla et al⁹ in a study of 108 individual donors for liver transplant (86 healthy donors and 22 patients with NAFLD) compared FIB-4 and magnetic resonance elastography (MRE) vs. liver biopsy to assess the fibrosis stages F0 vs. (F1-F4). Authors concluded that FIB-4 correlated well with biopsy (AUROC of 0.963). MRE performed even better than FIB-4 with an AUROC of 0.997. A FIB-4 cut-off of 1.0, showed sensitivity of 100% and specificity of 94.3% to rule out any fibrosis (F0 versus F1-F4). Authors further validate the cut off with MRE in a cohort of 239 patients with NAFLD. In this validation cohort study, FIB-4 with a cut-off of 1.0 was slightly inferior, with an AUROC of 0.843 (86.9% sensitivity 68.6% specificity). Authors concluded that a cut off of 1.0 performed well in young healthy living donors for liver transplant. Thus, various studies have shown different lower cut offs to predict fibrosis especially in F0 vs F1-F4 in Fatty Liver.

Disadvantages of FIB-4

FIB-4 is not sensitive in patients with diseases that cause platelet abnormalities especially thrombocytosis and an inverse AST/ALT ratio (e.g. muscle breakdown, alcohol abuse)

In our study, males >45yrs had mean FIB 4 cut off values more than their contraries. FIB 4 values increased with increase in grades of fatty liver but not statistically significant. Cut off values were lower in the study population than that reported in the west. Sensitivity, specificity, PPV and NPV were 51%,50%,34%,67% for FIB 4 in predicting fibrosis with US graded FL. (Grade 1,2,3)

Limitations of the study

The degree of fibrosis could not be correlated with fibro scan as data was not uniformly available for all the patients.

Number of patients in normal and Grade 1 FL were higher than other grades.

Conclusion:

FIB-4 score in our study is lower than that reported in the west with poor prediction for any grade of FL. More validation is required pertaining to non-invasive markers predicting fibrosis in NAFLD.

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308**Table 1: Descriptive analysis of age in study population (N=741)**

Parameter	Mean $\pm$ SD	Median	Minimum	Maximum	95% C. I	
					Lower	Upper
Age	48.21 $\pm$ 13.47	48.00	17.00	85.00	47.24	49.18

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310**Table 2: Descriptive analysis of age groups in the study population (N=741)**

Age Group	Frequency	Percentages
≤ 45 years	323	43.59%
> 45 years	418	56.41%

311

312**Table 3: Descriptive analysis of gender in the study population (N=741)**

Gender	Frequency	Percentages
Male	474	63.97%
Female	267	36.03%

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314**Table 4: Descriptive analysis of FIB 4 in study population (N=741)**

Parameter	Mean $\pm$ SD	Median	Minimum	Maximum	95% C. I	
					Lower	Upper
Fib -4 Score	1.3 $\pm$ 0.99	1.03	0.19	8.06	1.23	1.37

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318**Table 5: Descriptive analysis of fatty liver in the study population (N=741)**

Fibrosis	Frequency	Percentages
Normal	297	40.08%

Grade 1	293	39.54%
Grade 2	114	15.38%
Grade 3	37	4.99%

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324**Table 6: Comparison of mean fib -4 score across the study groups (N=741)**

Fibrosis	FIB -4 Score Mean $\pm$ SD	Mean difference	95% CI		P value
			Lower	Upper	
Normal	1.27 $\pm$ 1.05				
Grade 1	1.27 $\pm$ 0.84	0.01	-0.15	0.17	0.907
Grade 2	1.34 $\pm$ 1.13	0.07	-0.14	0.29	0.500
Grade 3	1.62 $\pm$ 1.13	0.35	0.02	0.69	0.038

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326**Table 7: Descriptive analysis of fibrosis in the study population (N=741)**

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Fibrosis	Frequency	Percentages
Normal	297	40.08%
Any fibrosis	444	59.92%

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329**Table 8: Predictive validity of Fib-4 score in predicting any fibrosis in the study population**
330**(N=741)**

Parameter	Value	95% CI	
		Lower	Upper
Sensitivity	48.82%	43.00%	54.66%
Specificity	47.30%	42.57%	52.06%
False positive rate	52.70%	47.94%	57.43%
False negative rate	51.18%	45.34%	57.00%
Positive predictive value	38.26%	33.34%	43.36%
Negative predictive value	58.01%	52.74%	63.15%
Diagnostic accuracy	47.91%	44.26%	51.57%
Positive likelihood ratio	0.93	0.74	1.074

Negative likelihood ratio	1.08	0.06	1.255
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Area	Std. Error	Asymptotic 95% CI for AUC		P value
		Lower Bound	Upper Bound	
0.509	0.033	0.445	0.572	0.790

332Threshold fib-4 score Cut off values: 0.72

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336Table 9: Predictive validity of Fib-4 score in predicting Severe fibrosis (grade 1 and more)
 337(N=444)

Parameter	Value	95% CI	
		Lower	Upper
Sensitivity	51.66%	43.39%	59.85%
Specificity	50.17%	44.34%	56.00%
False positive rate	49.83%	44.00%	55.66%
False negative rate	48.34%	40.15%	56.61%
Positive predictive value	34.51%	28.33%	41.10%
Negative predictive value	67.12%	60.51%	73.26%
Diagnostic accuracy	50.67%	45.94%	55.39%
Positive likelihood ratio	1.04	0.76	1.266
Negative likelihood ratio	0.96	0.06	1.177

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357**Figure 1: Mean error plot diagram of comparison of mean fib -4 score across the fibrosis**
358**group (N=741)**

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372**Figure 2: ROC analysis of predictive validity of fib-4 scores in predicting any fibrosis**
373**(grade 1,2 and 3) among the study population (N=741)**

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Diagonal segments are produced by ties.

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Area	Std. Error	Asymptotic 95% CI for AUC		P value
		Lower Bound	Upper Bound	
0.538	0.022	0.496	0.581	0.078

377