Original Article

Relationship of FIB-4 index with transient elastography in chronic hepatitis C patients having APRI ≥ 0.5 - ≤2 in a resource-limited setting in Pakistan

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Abstract

Objective: We aimed to study the extent of liver fibrosis in chronic hepatitis C patients with indeterminate APRI score of ≥ 0.5 - ≤2 (between higher and lower cut off value) and correlate it to transient elastography (TE) and FIB 4 index. **Method:** A cross-sectional study, 80 patients with CHC mono infection, APRI score ≥ 0.5 - ≤2 were interviewed from the cohort visiting the CHC program clinic at a tertiary care hospital in Karachi, Pakistan. Data were analyzed using STATA 14.0 and R 3.5.2 and SPSS 24.0 software according to their capabilities. **Result:** Of 80 patients, 50 (62.5%) were females and 30 (37.5%) were males with mean (±SD) ages of 41.73 (±11.5) years and 41.16 (±9.24) years respectively. The FIB 4 value among indeterminate APRI was reported as 1.47 (IQR 1.05-2.43). TE categories was reported: F0-F1 (n = 29; 36%), F1-F2 (n = 10; 12.5%), F2 (n = 9; 11.2%) F3 (n = 13; 16.2%), F3-F4 (n = 1; 1.2%) F4 (n = 18; 22.5%). FIB4 had a moderate positive correlation with TE while a weak positive correlation was found between APRI and TE (0.488, P < 0.0001 and 0.289, P < 0.001, respectively). TE was taken as a gold standard and compared with FIB4. The model constructed reported FIB4 as a good prediction for liver fibrosis with diagnostic accuracy 72%. **Conclusion:** The combination of two serum markers proves to be a low-cost noninvasive testing strategy for CHC patients having an indeterminate APRI score. By being readily accessible both biochemical scores can simplify liver assessment in lower middle-income countries (LMIC) and help family physicians to take appropriate decisions about treatment initiation with minimum delays.

Keywords: Chronic hepatitis C, FIB4 index, indeterminate APRI score, transient elastography

Background

Chronic hepatitis C infection (CHC) is one of the major public health problems worldwide leading to a huge economic burden on the health system.[1,2] In 2015, WHO (World Health Organization) reported approximately 71 million persons living with CHC infection worldwide and 399,000 deaths from cirrhosis or hepatocellular carcinoma caused by CHC.[3] To combat these overwhelming figures, WHO is working with health authorities in different countries to develop the hepatitis control program and to achieve hepatitis elimination by 2030.[4]

Pakistan is a low middle-income country (LMIC) that bears the second largest burden of CHC.[5] Due to fragmentation in the public health care system in the country, such as lack of consistency of financing and no national registry, one of the bottleneck to achieving CHC elimination goals is to identify active infection amongst the population at risk (through PCR

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testing) and linkage to care for further evaluation (to estimate severity of liver fibrosis) and treatment.[6] This hindrance has put a large number of patients at risk of being lost to follow up and presenting with advanced liver disease. In the past decade, the availability of Direct Acting Anti-viral agents (DAA) has been instrumental in reducing the cost of treatment and shifting management of HCV from specialists to generalists. Unfortunately, the expense and expertise required for the testing modalities including the determination of severity of liver fibrosis continue to be a hurdle at the primary care level.[7]

Assessing the degree of liver fibrosis is important as it dictates the choice of drug regimen and duration of treatment. The severity of liver damage can be assessed through a liver biopsy which is considered as a gold standard, interpreted using a metavir scoring system.[8] However, it is a resource-intensive procedure conducted at risk of complications such as infections and hemorrhage along with significant intra and interobserver variability.[9] Given potential risks associated with liver biopsy and unavailability of resources, clinical and public health specialists recommend the use of noninvasive methods for evaluating liver fibrosis.[10]

Noninvasive tests include radiological i.e., transient elastography (TE) and biochemical i.e., FIB 4 (fibrosis 4 index) and APRI (Aspartate Platelet Ratio Index) assessment which facilitates in providing an objective assessment without putting patients at risk of complication associated with liver biopsy.[11] TE, which measures the liver stiffness, has emerged as a reliable and validated screening tool for predicting advanced fibrosis with sensitivity and specificity of around 90%.[9] However, obesity and acute hepatitis are limitations in the accurate interpretation of TE.[12] TE is a good modality but requires specialist input, has a higher cost, and is not readily accessible in the developing world.[13]

APRI and FIB 4 are the most popular biochemical scores that use liver enzymes as variables. APRI is based on serum aspartate aminotransferase (AST) and platelets (PLT) while FIB 4 requires age, serum aspartate aminotransferase (AST), alanine transaminases, and platelets (PLT).[11] Both tests are consistent with each other in diagnosing advanced liver damage,[14] but their utility is limited for identifying the stage of liver damage within an indeterminate score (APRI $\geq 0.5 - \leq 2$ and FIB $4 \geq 1.45 - \leq 3.25$).[15] Nevertheless, concomitant use of both tests enables one to discriminate against the different stages as well as reduces the number of undiagnosed cases that are reported in using either test in isolation.[16] Recently, researches are conducted to define a threshold cut-off for both biochemical scores to diagnose severe liver fibrosis to defer TE.[7,17] FIB outperforms APRI, with a score of less than 1.08-1.2 as a threshold to exclude severe liver fibrosis found in different population.[11,13] Because of all the challenges associated with the assessment of liver fibrosis, WHO and EASL recommend using APRI and FIB-4 to guide monitoring and treatment decisions in HCV patients for a cost-effective care plan. APRI is taken as the initial tool for liver fibrosis determination and in patients falling within indeterminate APRI range, lab tests are coupled with TE.[18,19]

But the recent literature supports FIB 4 to have a higher negative predictive value (NPV) to exclude cirrhosis thus a better predictor to assess liver fibrosis.[20]

As TE is not readily accessible, the need of the time is to delve into a simplified testing strategy to estimate liver fibrosis for managing HCV infected patients at the primary care level. This study aimed to determine the association of indeterminate APRI score with FIB 4 score and TE.

Material and Method

A cross-sectional study nested within an ongoing CHC treatment clinic based at the outpatient department of a tertiary care hospital in Karachi, Pakistan.

The sample size was calculated to be 75 (5.4% margin of error), using open epi (https://www.openepi.com/SampleSize/SSPropor.htm) with the prevalence of CHC in Pakistan at 6%[21] and CI of 95.

A list of patients with indeterminate APRI scores was provided by the CHC treatment clinic team. Participants were patients with CHC mono-infection, treatment naïve, and clinically stable with no signs of liver decompensation. Patients with comorbidity were included in the study and were linked to specialist care for follow up. Those who consented to participate were interviewed during their regular follow up in the HCV treatment clinic. Interview (10–15 min) was conducted by the principal investigator and a trained staff nurse. Data were collected from October 2017 to August 2018.

The Questionnaire included patient demographics, medical history, clinical and laboratory data. Patient laboratory test values were accessed through the hospital electronic record. APRI and FIB 4 were calculated using original formulas[10] No additional tests were done for this study. As part of routine clinic protocol, limited resources allowed us to only refer CHC patients from with indeterminate APRI for TE.

The analysis is based on liver fibrosis as per TE scoring for CHC patients suggesting F0–F1 as no fibrosis (TE < 7), F1–F2 = presence of fibrosis (TE 7–8.5), F2 = mild fibrosis (TE 8.5–9.5), F3 = moderate fibrosis (TE 9.5–12.5), F3-F4 = severe fibrosis (TE 12.5–14) and F4 = cirrhosis (TE > 14).[22]

The study protocol was approved by the Interactive Research Development- Institute Review Board (IRD-IRB) - ID # IRD_IRB_2017_06_007.

Statistical analysis

Data were analyzed using R 3.5.2 and SPSS 24.0 software according to their capabilities. Mean ± SD/Median (IQR) was computed as appropriate for all the quantitative variables like age, APRI, and FIB4. All the categorical variables were presented as frequencies along with percentages. Independent sample *t* test/Mann–Whitney *U* test was applied as appropriate to assess

significant differences in various quantitative variables between both the genders. Chi-square test/Fisher exact test was applied as appropriate to assess the association between various categorical variables. Both univariate and multivariate ordinal regression was performed to build a fibrosis prediction model using SPSS. All the variables with $P < 0.25$ and of clinical and biological significance were included in the model. A manual step-wise regression analysis was done to eliminate all the insignificant variables. Using the final model, probabilities to predict liver fibrosis category were calculated using equation (1-6) (Refer to Appendix) and the category with the highest probability was deemed as predicted category for liver fibrosis. The diagnostic accuracy of the model was assessed through Nancy Obochowski's ordinal regression ROC analysis using R software. P value < 0.05 was considered statistically significant. The results were converted into a calculator applying maximum penalty (i.e. 1 for each category).

Results

A total of 952 patients were consulted in the HCV treatment clinic from October 2017 to August 2018 of which 352 (40.8%) were reported to have an in-determinant APRI score. Among 352 patients, a total of 80 (22.7%) patients were enrolled in this study.

The majority of the patients were females ($n = 50$; (62.5%), with no significant difference in age between both the genders (mean ± SD of 41.73 ± 11.5 in males vs. 41.16 ± 9.24 years in females, $P = 0.818$, Table 1). Most of the patients were married ($n = 74$, 92.5%), and more than half of the patients ($n = 43$, 53.8%) were unemployed [Table 2]. Moreover, only 24 (30%) patients reported addiction to substance abuse of which the majority were cigarette smokers ($n = 13$; 54.2%, Table 2).

No significant differences were observed in age and BMI between both genders. However, in a laboratory parameter, males were found to have higher hemoglobin, bilirubin, and serum creatinine in comparison to females [Table 1]. On the other hand, platelets and AST were higher in females as compared to males [Table 1].

A total of 26 patients reported having other comorbidities with no statistically significant association with gender (p = 0.292). Hypertension was the most common comorbidity ($n = 15$; 57.7%) followed by diabetes mellitus ($n = 8$; 30.8%) [Table 1].

Of a total of 80 participants, 68.8% ($n = 55$) had APRI score between 0.5-1, 16.2% ($n = 13$) had score 1.01 to 1.5 and 15% ($n = 12$) 1.51-to 2.

On assessment of TE categories 36% ($n = 29$) were F0-F1, F1-F2 ($n = 10$; 12.5%), F2 ($n = 9$; 11.2%) F3 ($n = 13$; 16.2%), F3-F4 ($n = 1$; 1.2%) F4 ($n = 18$; 22.5%)

Furthermore, it was observed that more than half of patients with APRI score 0.5 to 1.0 had severe fibrosis in TE with

56.4% ($n = 31/55$) reported F3-F4 and 12.7% ($n = 7$) had F4. Also, in patients who had APRI score 1.5 to 2, TE reported F4 in two-third of the patients ($n = 8$, 66.7%) followed by F3 ($n = 1$, 8.3%) F2 ($n = 2$, 16.6%) and F0-F1 ($n = 1$, 8.3%).

The FIB 4 value among indeterminate APRI was reported as 1.47 (IQR 1.05-2.43). The study found that 40% ($n = 32$) had indeterminate FIB 4 (>1.45-<3.25). However $n = 39$; 48.7% patients were found to have scores < 1.45 ruling out significant fibrosis and $n = 9$; 11.5% patients had FIB 4 > 3.25 suggestive of significant liver fibrosis.

When all three tests were compared, FIB4 had a moderate positive correlation with TE while a weak positive correlation was found between APRI and TE (0.488, $P < 0.0001$ and 0.289, $P < 0.001$, respectively).

On further analysis of the range of APRI and FIB-4 scores with TE categories, there was a significant difference in FIB-4 values amongst the TE categories ($P < 0.0001$, Table 3). However, an overlap in FIB 4 range among different TE categories was observed, which limited the use of FIB 4 to diagnose the severity of liver fibrosis [Table 3].

When comorbidities were compared with the status of liver damage, significant fibrosis (F3 & F4) was noted in a patient with diagnosed diabetes mellitus and hypertension ($P = 0.01$, Table 4)

Diagnostic Accuracy of FIB-4

Ordinal regression analysis was done including age, gender, BMI, and FIB4 predictor variables that had $P < 0.25$ in the univariate analysis. Few variables (i.e. substance use and comorbid conditions) could not be included due to the small sample size within each category.

Transient Elastography (TE) was taken as the gold standard in this study and the model was built after removing all the insignificant variables through the step-wise method. The final model included the FIB4 only. Using this model, predicted category probabilities were calculated using equation (1-6) (See Appendix A) and the category with the highest probability was deemed as a predicted category for liver fibrosis.

Results showed that the model constructed has a good prediction for liver fibrosis with an accuracy of 72%. Moreover, it is evident from the pairwise comparison that the model is a decent prediction model of higher categories of liver fibrosis [Tables 5 and 6]. Based on the results, a calculator was designed to assist primary care physicians in the decision regarding whether or not to refer patients for TE concerning the given limitation of FIB 4. Supplement 1 (Hyperlink of the calculator).

Table 1: Differences in demographic characteristics and lab results between both the genders				
	Gender		Total	*P*
	Male	Female		
Age in years				
Mean±SD	41.7±41.2	41.2±9.2		0.818[‡]
Min-Max	21-60	21-63	21-63	
Median (IQR)	40 (34-52)	42 (34.5-47.25)	42 (34-50)	
BMI				
Mean±SD	25.4±26.2	26.2±5.3	25.90±5.39	0.377[†]
Min-Max	16.54-41.87	16.4-36.92	16.40-41.87	
Median (IQR)	23.9 (22.145-27.825)	25.94 (22.56-29.925)	24.7 (22.46-29.17)	
Hb				
Mean±SD	15.3±12.5	12.5±1.7	13.56±2.08	0.000**,[‡]
Min-Max	12.6-18.4	7.4-15.2	7.4-18.4	
Median (IQR)	15.15 (14.2-16.2)	13 (11.1-13.7)	13.65 (12.35-15.10)	
Platelets				
Mean±SD	192.3±240.6	240.6±71.3	222.52±70.10	0.004*,[†]
Min-Max	52-270	72-505	52--505	
Median (IQR)	194.5 (150.75-246)	235 (197-280.25)	225.50 (176.25-259.50)	
HbA1C				
Mean±SD	5.7±5.6	5.6±1.2	5.612±1.05	0.175[†]
Min-Max	4.1-7.9	3.7-11.6	3.70-11.60	
Median (IQR)	5.5 (5.3-5.9)	5.3 (5.075-5.725)	5.4 (5.15-5.8)	
Bilirubin				
Mean±SD	0.8±0.6	0.6±0.26	0.64±0.327	0.007*,[†]
Min-Max	0.18-2.05	0.08-1.611	0.08-2.05	
Median (IQR)	0.685 (0.5-0.82)	0.52 (0.44-0.66)	0.56 (0.46-0.74)	
ALT				
Mean±SD	105.8±77.2	77.2±31.0	87.9±48.4	0.091[†]
Min-Max	24-282	15-153	15-282	
Median (IQR)	87.5 (57-139.25)	72.5 (52.5-94.5)	72.5 (52.5-94.5)	
AST				
Mean±SD	68.1±83.4	83.4±31.8	77.7±29.8	0.027*,[†]
Min-Max	39-127	39-169	39-169	
Median (IQR)	60 (49.25-82.5)	75 (62-100.5)	71 (54.2-92)	
Serum creatinine				
Mean±SD	0.9±0.7	0.7±0.12	0.75±0.17	0.000**,[‡]
Min-Max	0.54-1.19	0.46-1.01	0.46-1.2	
Median (IQR)	0.895 (0.8-0.99)	0.66 (0.58-0.75)	0.72 (0.6-0.89)	

Independent sample *t*-test, [†]Mann-Whitney U test, [‡]Multiple response Chi-square test

Discussion

Assessment of severity of liver fibrosis in CHC patients before therapy is essential.

Recognizing patients with severe fibrosis (F3) and advanced fibrosis/cirrhosis (F4) is pivotal for decision on duration and choice of treatment as well as their prognosis post therapy. In order to achieve HCV elimination goals by 2030, addressing delays associated with assessing severity of liver cirrhosis should be prioritized.[23]

Transient Elastography is now accepted as an alternative gold for hepatic fibrosis assessment in HCV patients by many guidelines. However, a high cost associated with the test makes accessibility difficult in low and middle-income countries.[24] To prioritize patients for treatment, concomitant use of FIB 4 in the patient falling in indeterminate APRI can help family physicians in decision making. This could further decrease the number of appointments as well as secondary care/specialist referrals for liver assessment and make appropriate use of DAA regime possible at the primary care level.[25]

This study found that concomitant use of FIB4 could diagnose the severity of liver fibrosis in approximately 70% of patients falling within indeterminate APRI scores. The combination of the biochemical scores decreases the number of undiagnosed cases within the indeterminate range and can ease the gap between accurate liver assessment and treatment initiation. These findings are consistent with other studies where authors have shown the application of all three tests to assess liver fibrosis.[16,24] Additional testing is seldom required, the use of FIB 4 and APRI could be beneficial for physicians in settings where TE is not available. FIB 4 includes age and ALT level, therefore it's found

Table 2: Characteristics of study participants	
Demographics	*n*%
Gender	
Male	30 (37.5)
Female	50 (62.5)
Marital Status	
Married	74 (92.5)
Single	5 (6.3)
Widow	1 (1.3)
Occupation	
House wife	43 (53.8)
Service and sale workers	8 (10)
Craft and related trade workers	7 (8.8)
Clerks	4 (5)
Plant and machine operators and assembles	6 (7.5)
Elementary occupations	4 (5)
Technicians and associate professionals	4 (5)
Others	4 (5)
Ethnicity	
Punjabi	29 (36.3)
Urdu speaking	26 (32.5)
Sindhi	9 (11.3)
Pakhtoon	3 (3.8)
Others	12 (15.1)
Not recorded	1 (1.3)
Substance abuse	
Cigarette smoking	13 (54.2)
Ghutka	5 (20)
Niswar	3 (12.5)
Beetle nuts	3 (12.5)
Total	24 (100)

to be superior to APRI in diagnosing advanced cirrhosis.[26] When compared with TE, FIB4 has its limitation to differentiate between two consecutive TE categories, thus further evaluation in such patients is warranted.

Previous studies have reported severity in liver fibrosis with aging[13] but we found no significant difference in liver fibrosis with increasing age in patients with indeterminate APRI. Furthermore, the literature suggests that estrogen causes less severity of fibrosis,[27] whereas in our study majority patients were females in fertile age, however, no difference in the severity of liver fibrosis was observed among genders. One interesting finding of this study was a raised ALT and platelets observed in females compared to males; it is hypothesized to be that could be due to insulin resistance and hepatosteatosis, which is evident in previous literature,[28,29] however, this is one limitation of the study as testing for metabolic syndrome (fasting lipid profile, FBS, etc.) was not incorporated, Further studies are recommended to get a clear association with this aspect.

Moreover, BMI influence on liver fibrosis is debatable as few researchers found a positive correlation and others had inconclusive results.[13,30] We observed no significant difference in BMI among genders, and when the ordinal regression was applied BMI did not present as a predictor variable. Nevertheless, South Asians are vulnerable to insulin resistance therefore patients with even lower BMI are at substantial risk to develop NAFLD, Co-existing

liver disease may afflict liver fibrosis. Non-alcoholic fatty liver disease (NAFLD) being the most common liver disease in the western world, influences 18% of the Pakistani population.[31] Seven patients (3 females and 4 males) in this study group were observed to have fat deposition on ultrasound of which 4 patients were found to have comorbid conditions and TE score in the cirrhotic range.

Chronic medical conditions and medication contributes to liver fibrosis.[13] A total of 26 patients reported comorbid conditions, of which 7 hypertensive and 5 patients with type 2 diabetes mellitus had severe fibrosis [Table 4]. Due to a very small sample size of patients with comorbidities, an inference cannot be made, but that CHC patient with comorbid conditions although controlled on medication must be referred for TE as they are at two-three fold risk of developing F3-F4 liver fibrosis as previously studied.[32]

This study is unique as it assessed the utility of FIB 4 in the patient falling in indeterminate APRI score to formulate a simplified testing strategy for clinicians practicing in a resource-limited setting. This could minimize the need for expensive testing, specialist referrals as well as decrease the gap between detection and initiation of hepatitis C treatment. This will enable family physicians to confidently initiate the appropriate DAA regimen for their patients. The calculator devised can also help family physician to decide whether to treat or refer CHC patients, nevertheless, clinical history and examination for risk factors of liver fibrosis remain essential concomitantly. Therefore this calculator has limited use in liver fibrosis assessment for patients with comorbid conditions or co-existing liver disease as they are more susceptible to liver fibrosis, hence should not be used in these patients.

As the study was nested in an ongoing HCV program, there was a limited number of TE performed. We were unable to overcome a small sample size that has been mentioned in previous studies as well. The majority of patients with the comorbid condition were found to have an APRI score between 0.5-1 but TE reported severe fibrosis (F3) or cirrhosis (F4) but we were not able to further analyze due to limited sample size within each TE category.

For future studies, a larger sample size should be considered to evaluate indeterminate APRI with FIB 4 and TE. We suggest the validation of this tool as well as the feasibility of different populations. We further recommend studies to determine liver fibrosis in CHC patients having the comorbid and co-existing, non- infective liver disease (NAFLD).

We conclude that use of concomitant FIB 4 in CHC patients falling in indeterminate APRI score addresses time delays in diagnosing and treatment initiation. It's a cheaper option, accessible through primary care clinic/system and useful for patients where TE is not available, indicated or will give false results (morbid obesity, narrow intercostal spaces).

This would empower family physicians to make the appropriate decision about treatment regimen and duration, enables shift

Table 3: Difference in demographic variables, APRI, FIB4 and time lapse in seeking treatment according to status of liver cirrhosis

	Transient Elastography results						Overall
	<7.0 (F0-F1) No fibrosis	7.0-<8.5 (F1-F2) Presence of fibrosis	8.5-<9.4 (F2) Mild fibrosis	9.5-<12.5 (F3) moderate fibrosis	12.5-<14 (F3-F4) Severe fibrosis	>=14 (F4) Cirrhosis	
Age in years							
n	28	5	6	21	3	17	80
Mean±SD	34.2±8.7[e,f]	42±4	49.2±11.6	43±7.7	43.3±8	47.9±9.1	41.4±10.1
Min-Max	21-57	38-46	27-59	30-58	35-51	26-63	21-63
Median (IQR)	33.5 (29.3-37.5)	42 (38-46)	50.5 (44.3-58.3)	43 (37-49.5)	44 (35-0)	48 (43.5-53.5)	42 (34-50)
P	0.000**,[‡]						
BMI							
n	23	5	6	16	3	17	70
Mean±SD	24±4.8	26.3±6.9	23.9±6.7	25.9±4	28.8±0.6	28.6±6.1	25.9±5.4
Min-Max	16.5-35.9	20.3-33.8	17.9-32.8	16.4-33.3	28.3-29.6	19.1-41.9	16.4-41.9
Median (IQR)	23 (21.2-26.6)	22.8 (20.6-33.8)	21.1 (18.6-32)	25.7 (23.7-28)	-	26.7 (24-33.2)	24.7 (22.5-29.2)
P	0.149[‡]						
APRI							
n	28	5	6	21	3	17	80
Mean±SD	0.8±0.3	1.2±0.6	0.9±0.3	0.8±0.3	1±0.2	1.3±0.5	0.95±0.42
Min-Max	0.5-1.6	0.6-1.8	0.5-1.2	0.5-1.7	0.8-1.3	0.6-1.9	0.50-1.9
Median (IQR)	0.7 (0.5-1)	1.3 (0.7-1.8)	1 (0.5-1.2)	0.8 (0.6-1)	-	1.5 (0.7-1.8)	0.8 (0.61-1.2)
P	0.185[†]						
FIB 4							
n	28	5	6	21	3	17	80
Mean±SD	1.1±0.4	2±1	2.3±1.4	1.9±1	2±0.5	3±1.4	1.9±1.2
Min-Max	0.5-2.5	1.2-3.3	0.6-4.3	1.1-5.7	1.6-2.5	1.1-6.6	0.47-6.6
Median (IQR)	1 (0.8-1.3)	1.3 (1.2-3.1)	2.1 (1.1-3.9)	1.6 (1.2-2.3)	-	3 (2-3.6)	1.47 (106-2.4)
P	0.000**,[†]						
Time lapse in seeking treatment (months)							
n	25	4	6	21	3	12	71
Mean±SD	31.1±42.1	17.3±13.9	58±62.3	26.4±30.4	86±112.9	44±47.7	35.7±45.8
Min-Max	0.5-144	3-36	12-144	1-108	12-216	6-144	1-216
Median (IQR)	12 (6-33)	15 (5.2-31.5)	24 (12-135)	12 (4.5-36)	-	24 (6-72)	12 (6-36)
P	0.412[†]						

**P<0.0001, [†]Mann-Whitney *U*-test, [‡]One-Way ANOVA. For each significant pair, the key of the category (a=no fibrosis, b=presence of fibrosis, c=mild fibrosis, d=moderate fibrosis, e=severe fibrosis and f=liver cirrhosis) appears in the superscript

Table 4: Association of comorbidities and addiction with status of liver fibrosis

	Transient Elastography results						Total
	<7.0 (no fibrosis)	7.0-<8.5 (presence of fibrosis)	8.5-<9.4 (mild fibrosis)	9.5-<12.5 (moderate fibrosis)	12.5-<14 (severe cirrhosis)	>=14 (liver cirrhosis)	
Comorbid; n (%)							
Diabetes Mellitus (DM)	0 (0)	0 (0)	0 (0)	2 (25)	1 (12.5)	5 (62.5)	8 (100)
Mental Health Illness (such as depression)	2 (50)	0 (0)	0 (0)	2 (50)	0 (0)	0 (0)	4 (100)
Hypertension	0 (0)	0 (0)	0 (0)	7 (46.7)	1 (6.7)	7 (46.7)	15 (100)
Tuberculosis	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)
Blood disorder (Thalassemia, sickle cell disease)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)	1 (100)
Other	2 (28.6)	1 (14.3)	0 (0)	2 (28.6)	0 (0)	2 (28.6)	7 (100)
P				0.010*,[‡]			
Addiction; n (%)							
Cigarette Smoking	5 (38.5)	0 (0)	1 (7.7)	2 (15.4)	0 (0)	5 (38.5)	13 (100)
Ghutka	2 (33.3)	0 (0)	1 (16.7)	1 (16.7)	0 (0)	2 (33.3)	6 (100)
Niswar	2 (66.7)	0 (0)	0 (0)	1 (33.3)	0 (0)	0 (0)	3 (100)
Chaliya	0 (0)	0 (0)	0 (0)	2 (66.7)	0 (0)	1 (33.3)	3 (100)
P				0.702[‡]			

*P<0.05, [‡]Multiple response Chi-square test, [†]Chi-square test

Table 5: Ordinal regression analysis					
	Estimate	Std. error	*P*	95% Confidence Interval	
				Lower Bound	Upper Bound
Threshold					
No fibrosis	1.366	0.469	0.004*	0.447	2.285
Presence of fibrosis	1.717	0.479	0.000**	0.779	2.656
Mild fibrosis	2.097	0.495	0.000**	1.127	3.068
Moderate fibrosis	3.564	0.606	0.000**	2.376	4.752
Severe fibrosis	3.854	0.632	0.000**	2.615	5.093
Liver cirrhosis	Ref				
Location					
FIB4	1.218	0.257	0.000**	0.714	1.722

Table 6: Diagnostic accuracy of the prediction model		
Pair	Estimate	Standard Error
1 vs 2	0.68	0.13
1 vs 3	0.73	0.12
1 vs 4	0.68	0.06
1 vs 5	0.80	0.17
1 vs 6	0.90	0.05
2 vs 3	0.52	0.18
2 vs 4	0.55	0.17
2 vs 5	0.50	0.23
2 vs 6	0.66	0.16
3 vs 4	0.58	0.14
3 vs 5	0.53	0.20
3 vs 6	0.67	0.14
4 vs 5	0.59	0.16
4 vs 6	0.78	0.07
5 vs 6	0.77	0.10
Overall	0.72	0.03

from specialist to generalist care, which is essential in case of managing an epidemic. Moreover, the use of both biochemical scores would benefit primary care physicians to identify need for HCC surveillance/variceal banding in patients before or after HCV treatment that requires referral to secondary care. Eventually, the health care system would improve with simplification of disease management pathways and decrease the burden on secondary and tertiary care hospitals.

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Ethical statement

Interactive Research Development- Institute Review Board (IRD-IRB) reviewed the protocol for human subjects and issued approval to the study ID # IRD_IRB_2017_06_007 on 19th December 2017.

Subjects have given verbal consent to participate in this study.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient (s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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APPENDIX A

Equations:

$$Prob_{cat1} = \frac{exp\ exp\ (eta\ 1)}{1 + exp\ exp\ (eta\ 1)} \tag{1}$$

$$Prob_{cat2} = \left(\frac{exp\ exp\ (eta\ 2)}{1 + exp\ exp\ (eta\ 2)}\right) - Prob_{cat1} \tag{2}$$

$$Prob_{cat3} = \left(\frac{exp\ exp\ (eta\ 3)}{1 + exp\ exp\ (eta\ 3)}\right) - Prob_{cat1} - Prob_{cat2} \tag{3}$$

$$Prob_{cat4} = \left(\frac{exp\ exp\ (eta\ 4)}{1 + exp\ exp\ (eta\ 4)}\right) - Prob_{cat1} - Prob_{cat2} - Prob_{cat3} \tag{4}$$

$$Prob_{cat5} = \left(\frac{exp\ exp\ (eta\ 5)}{1 + exp\ exp\ (eta\ 5)}\right) - Prob_{cat1} - Prob_{cat2} - Prob_{cat3} - Prob_{cat4} \tag{5}$$

$$Prob_{cat6} = 1 - Prob_{cat1} - Prob_{cat2} - Prob_{cat3} - Prob_{cat4} - Prob_{cat5} \tag{6}$$

$$eta_i = a_i + 1.218 * FIB4 \qquad\qquad i = 1\ to\ 5$$

where, *a=threshold value for each category [Tables 5 and 6]*

Supplement 1

Calculator

FIB4 value	2.25
Expected level of fibrosis	**Moderate fibrosis**