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Assessment of Liver Fibrosis Using Real Time Elastography and FIB 4 Score in Comparison to Liver Biopsy in Chronic HCV Egyptian Patients

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ABSTRACT

Background: HCV is one of the etiologies causing liver fibrosis through direct deposition of extracellular matrix in the liver as a response to wound healing to compensate for the injury

Aim of this study: assessment of liver fibrosis using sonography-based real time elastography (RTE) and FIB 4 score in comparison to liver biopsy for assessment of the stage of liver fibrosis in chronic Egyptian HCV patients.

Patients and Methods: one hundred sixteen (116) patients with HCV were enrolled in the study from 2014-2015 in the National Hepatology and Tropical Medicine Research Institute Outpatient Clinics, Cairo, Egypt.

Results: the mean RTELFI of the studied group was 2.68 ± 0.74. The correlation between different fibrosis stages, FIB-4 and RTELFI showed significant relations between the degree of hepatic fibrosis and FIB-4 with p = 0.001 & RTELFI with p <0.001. Positive correlation was detected between fibrosis stage, FIB-4 and RTELFI. At a cutoff value of 3.26 for RTELFI, the test had a sensitivity of 72% and a specificity of 90% in detecting advanced fibrosis with AUC = 0.791 (95% confidence interval 0.649-0.933).At a cutoff value of 2.26 for FIB-4 index, the test had a sensitivity of 50% and a specificity of 87% in detecting advanced fibrosis with AUC =0.797 (95% confidence interval 0.683-0.911).

Conclusion: RTE has excellent accuracy for F4 and F≥3 and is superior to FIB-4.

Keywords: RTE, FIB4, HCV.

INTRODUCTION

HCV is one of the etiologies causing liver fibrosis through direct deposition of extracellular matrix in the liver as a response to wound healing to compensate for the injury. Liver fibrosis, whatever the degree of it, may be associated with worse outcomes in the context of HCV infection[(1)].

Liver biopsy has been always considered as the gold standard method of detecting degree of fibrosis. But, because of the associated risk of bleeding, there was refusal by many patients. Also, the sampling bias, and the variability in reading between pathologists[(2)]. Other non-invasive methods were introduced into the research field in a trial to find an easier way to judge the degree of fibrosis and in the same way avoid the previous disadvantages of liver biopsy[(3)].

FIB-4 and real time elastography were introduced in the last decade among many other diagnostic tools as APRI score, fibro-test, MRI elastography to help as non-invasive methods to detect the degree of liver fibrosis. The FIB-4 is a used equation for evaluating liver fibrosis, based on age, AST, ALT and platelet count. It was initially invented in the APRICOT study (AIDS Pegasys Ribavirin International Coinfection Trial) to evaluate the presence of liver fibrosis in HIV/HCV co-infected patients. At a cut-off of <1.45 in the validation set, the negative predictive value to exclude advanced fibrosis (stage 4-6) was 90% with a sensitivity of 70%. A cutoff of>3.25 had a positive predictive value of 65% and a specificity of 97%[(4)]. The problem with FIB-4is that it carries the risk of overestimating the degree of fibrosis due to the effect of necroinflammatory activity on transaminases[(5)].

Transient hepatic elastography (TE) is a simple non-invasive method that is used to evaluate liver stiffness, depending on uni-dimensional transient elastography, a technique that uses elastic waves and low frequency ultrasounds (50 Hz). TE was validated in many chronic liver diseases that are not due to viral infection[(6)]. In both adults and children, it was, initially projected and then validated in patients with chronic hepatitis C[(7)].

Several studies have emphasized the presence of a significant correlation between TE and fibrosis stage, as assessed by the METAVIR scoring system[(7)].

The old RTE module depended on compression by the operator to transmit a strain within the liver parenchyma then a strain profile is to be calculated along the compressed area. Nowadays, an automatic strain is generated by the heartbeat. In order to interpret the results to fibrosis indexes, a new interpretation

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have been developed based upon the analysis of the histogram[8].

Aim of the study:

The aim of this study is to validate the FIB-4 and Real Time Elastography Liver Fibrosis Index (RTELFI) in detecting fibrosis when compared to liver biopsy in chronic HCV patients.

PATIENTS AND METHODS

Patients:

This prospective study included 116 patients with chronic HCV as diagnosed by seropositivity for HCV antibodies and HCV RNA by real time PCR. Those patients were coming for treatment by sofosbuvir and ribavirin or Pegylated intereferon (IFN), sofosbuvir and ribavirin in National Hepatology and Tropical Medicine Research Institute Outpatient Clinics over the period from December 2014 to June 2015.

The study was approved by the Ethical Committee for the National Control of Viral Hepatitis of the Ministry of Health.

An informed consent including the study procedures and approved the usage of blood sampling and possible data application in future research was taken from all patients.

We included patients ≥18 years old with WBC >3500/mm, platelet count >75, prothrombin time <2 seconds above upper limit of normal, direct bilirubin 0.3 mg/dl or within 20% of upper limit of normal. Besides, fasting blood sugar within upper limit of normal, albumin >3.5gm/dl, normal serum creatinine and TSH, HBsAg negative, Anti-nuclear antibody (ANA) titre <1: 40, HBA1C <8.0 in case of diabetes and alpha fetoprotein <100 were included.

Patients with any other liver disease or decompensated liver cirrhosis were excluded from the study. Pregnant and lactating females were excluded.

Those with hypersensitivity to IFN or Ribavirin and those with poorly controlled DM were also excluded. No patients with organ transplantation were enrolled in the study.

Patients were subjected to full history taking and complete clinical assessment. Routine laboratory investigations were done (CBC, serum bilirubin, ALT, AST, alkaline phosphatase, serum albumin, INR, Serum creatinine, HBsAg, quantitative PCR for HCV, ANA and glucose profile)**.**

Methods:

Investigated markers:

1. FIB 4 Score*:* Age (years) x AST level (U/L)/ [platelet count (10^9/L) x √ALT level (U/L)].

- **2. Real-time tissue elastography:** RTE was performed by using HI VISION Preirus (Hitachi Medical, Tokyo, Japan) with a linear probe (EUP-L52; central frequency, 5.5 MHz).

3. Ultrasound guided liver biopsy:

- **3.1. Technique of liver biopsy:** Percutaneous liver biopsies were obtained using MedaxPoggiorusco, Italy, 16 G X 200 mm single step biopsy needle. Ultrasonic guidance was used.
- **3.2. Histological evaluation:** Percutaneous liver biopsies were fixed in 10% neutral formalin, processed then embedded in paraffin, and cut at 5 microns thickness. Histological sections were stained with haematoxylin and eosin and Masson trichrome stains. All biopsy specimens were analyzed by an experienced pathologist in Pathology Department, National Hepatology & Tropical Medicine Research Institute, who was blinded of clinical and laboratory data of the patients.

Liver fibrosis staging and activity grading was evaluated according to the METAVIR[2] scoring system.

- **Fibrosis was staged on a 0–4 scale as follows:**
- F0, no fibrosis;
- F1, portal fibrosis without septa;
- F2, portal fibrosis with rare septa;
- F3, numerous septa without cirrhosis;
- F4, cirrhosis.
- **- Activity (A) was graded as follows:**
- A0, no histologic necroinflammatory activity;
- A1, mild activity;
- A2, moderate activity;
- A3, severe activity.

Statistical analysis:

Analysis of data was performed using SPSS 21 for Windows. Description of variables was presented as follows:

Description of numerical variables was in the form of mean, standard deviation (SD), Median, 25^{th} and 75^{th} percentiles.

Description of categorical variables was in the form of numbers (No.) and percent (%).

Comparison between quantitative variables was carried out by student T-test of two independent samples. Repeated measures Analysis of Variance (ANOVA) test was used when comparing between more than two groups of independent variables. Results were expressed in the form of p-values. Comparison between qualitative variables was carried out by Chi-Square test (X^2).

Binary correlation was carried out by Pearson correlation test or Spearman correlation test in case of

ordinal variables. Results were expressed in the form of correlation coefficient (r) and p-values.

The following points were the accepted guidelines for interpreting the correlation coefficient:

0 indicated no linear relationship.

+1 indicated a perfect positive linear relationship: as one variable increases in its values, the other variable also increases in its values via an exact linear rule.

-1 indicated a perfect negative linear relationship: as one variable increases in its values, the other variable decreases in its values via an exact linear rule.

Receiver Operating Characteristic (ROC) curves were graphed to determine appropriate cutoff points of RTELFI, RTEAREA and FIB-4 score in predicting stage of liver fibrosis that give optimal sensitivity and specificity.

The significance of the results was assessed in the form of P-value that was differentiated into:

P-value >0.05: Non-significant.

P-value ≤0.05: Significant.

RESULTS

The histological findings in liver biopsies of studied patients regarding necroinflammatory score were as follows; 104 (89.7%) were A1; 10 (8.6%) were A2 and 2 (1.7%) were A3.As regards the fibrosis score; 2 (1.7%) were F0; 69 (59.5 %) were F1; 27 (23.3%) were F2; 15 (12.9%) were F3 and 3 (2.6%) were F4. The mean RTE LFI of the studied group was 2.68±0.74.

The correlation between different fibrosis stages, FIB-4 and RTELFI showed significant relations between the degree of hepatic fibrosis and FIB-4 with p-value of 0.001& RTELFI with p-value <0.001.

There were matched results between different stages of liver fibrosis assessed by liver biopsy and the readings of RTELFI, the mean RTELFI was 1.48 at F0; 2.48 at F1; 2.75 at F2; 3.38 at F3 and 3.93 at F4. There were matched results between different stages of liver fibrosis assessed by liver biopsy and FIB-4 index results. The mean FIB-4 was 1.24 at F0; 1.38 at F1; 1.63 at F2; 2.50 at F3 and 3.58 at F4.

The results were matched between advanced stages of liver fibrosis assessed by liver biopsy and the readings of RTELFI. The mean RTELFI was 2.54 at <F3 and 3.47 at ≥F3. In addition, there was a match between advanced stages of liver fibrosis assessed by liver biopsy and FIB-4 index results. The mean FIB-4 was 1.44 at < F3 and 2.68 at ≥F3.

The binary correlation between RTELFI, FIB-4 and categorical variables showed a weak positive linear correlation between RTELFI and necro-inflammatory score. A moderate positive linear correlation was noticed between RTELFI and fibrosis score and a moderate positive linear correlation was noticed between FIB-4 and both necroinflammatory score and fibrosis score.

The scattered plot diagrams showed positive linear correlation between liver fibrosis degrees and RTELFI, positive linear correlation between liver fibrosis degrees and FIB-4, and positive linear correlation between RTELFI and FIB-4. The ROC curve was graphed to determine the appropriate cutoff points of RTELFI and FIB-4 score in predicting stage of liver fibrosis that give optimal sensitivity and specificity. The positive actual state is determining the cutoff value at which advanced fibrosis (≥ F3) could be determined.

At a cutoff value of 3.26 for RTELFI, the test had a sensitivity of 72% and a specificity of 90% in detecting advanced fibrosis with AUC = 0.791 (95% confidence interval 0.649-0.933). At a cutoff value of 2.26 for FIB-4 index, the test had sensitivity of 50% and a specificity of 87% in detecting advanced fibrosis with AUC =0.797 (95% confidence interval 0.683-0.911).

Table (1): Baseline demographic numerical data of study patients

Variable	Mean	Standard deviation
Age/years	40.72	±10.45
Weight/Kg	75.97	±12.73
Height/cm	166.24	±9.56
BMI (Kg/m^2)	27.54	±4.43

Table (**1**) showed the baseline demographic data of the whole study patients. The mean age was 40.72±10.45 years, the mean body weight was 75.97±12.73 Kg, the mean height was 166.24±9.56 cm and the mean BMI was27.54±4.43Kg/m^2.

Table (2): Sex distribution among studied patients

Variable	Frequency	%
Male	68	58.6
Female	48	41.4
Total	116	100

Table (**2**) showed the sex distribution among studied population, 68(58.6%) were males while 48(41.4%) were females.

Table (3): Liver biopsy grading among studied patients

Variable		Frequency	%
Necro-inflammatory score	A1	104	89.7
	A2	10	8.6
	A3	2	1.7
Fibrosis Score	F0	2	1.7
	F1	69	59.5
	F2	27	23.3
	F3	15	12.9
	F4	3	2.6

Table **(3)** showed the summary of histological findings in liver biopsies of studied patients. Regarding necroinflammatory score, 104 (89.7%) were A1, 10 (8.6%) were A2 and 2 (1.7%) were A3. As regards the fibrosis score, 2 (1.7%) were F0, 69 (59.5 %) were F1, 27 (23.3%) were F2, 15 (12.9 %) were F3 and 3 (2.6%) were F4.

Table (4): Correlation between RTELFI, FIB-4, necroinflammatory score and fibrosis score

Variable		RTELFI	FIB-4
Necro-inflammatory score	Pearson Correlation "r" "r"	0.216*	0.382**
	Sig. (2-tailed)	0.020	0.000
Fibrosis Score	Pearson Correlation "r"	0.434**	0.396**
	Sig. (2-tailed)	0.000	0.000

Table (**4**) showed the binary correlations between RTELFI, FIB-4 and categorical variables. A weak positive linear correlation was noticed between RTELFI and necroinflammatory score. A moderate positive linear correlation was noticed between RTELFI and fibrosis score. A moderate positive linear correlation was noticed between FIB-4 and both necroinflammatory score and fibrosis score.

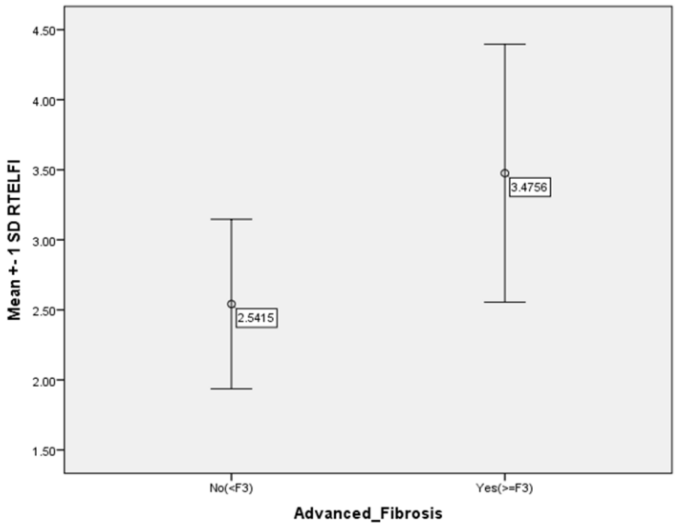

Figure (1): Relation between advanced fibrosis and RTELFI

Figure (**1**) showed matched results between advanced stages of liver fibrosis assessed by liver biopsy and the readings of RTELFI. The mean RTELFI was 2.54 at <F3 and 3.47at ≥F3.

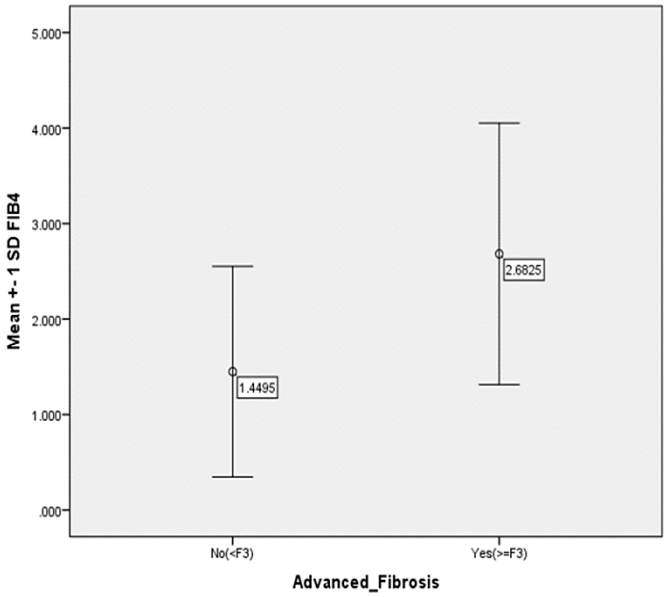

Figure (2): Relation between advanced fibrosis and FIB-4 index

Figure (**2**) showed matched results between advanced stages of liver fibrosis assessed by liver biopsy and FIB-4 index results. The mean FIB-4 was 1.44 at <F3 and 2.68 at ≥F3.

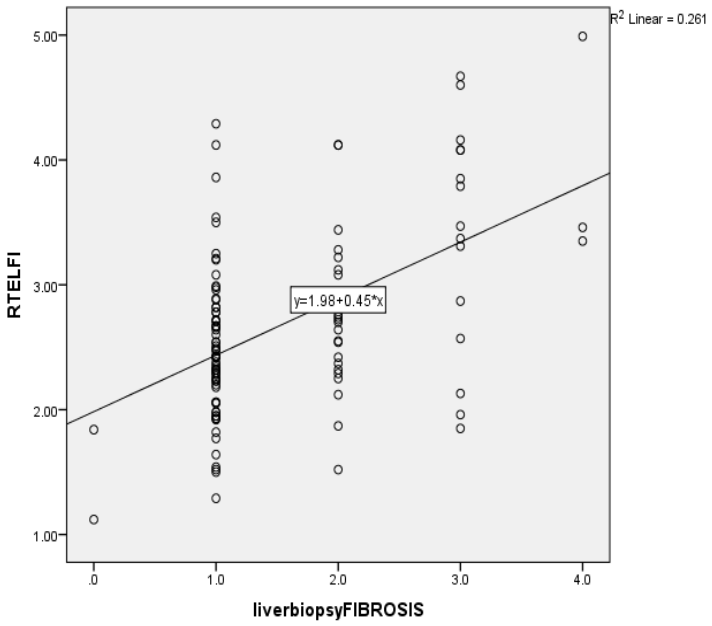

Figure (3): Correlation between different fibrosis stages and FIB-4

Figure **(3)** is a scattered plot diagram. It showed positive linear correlation between liver fibrosis degrees and FIB-4.

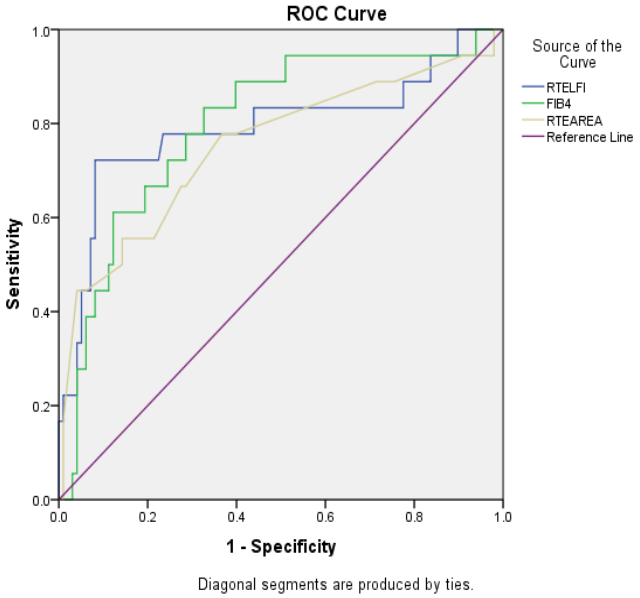

Figure (4): Receiver operating characteristic (ROC) curve for prediction of advanced fibrosis (≥F3)

Figure (**4**) showed the ROC curve graphed to determine appropriate cutoff points of RTELFI and FIB-4 score in predicting stage of liver fibrosis that give optimal sensitivity and specificity. The positive actual state is determining the cutoff value at which advanced fibrosis (≥F3) could be determined. At a cutoff value of 3.26 for RTELFI, the test had a sensitivity of 72% and a specificity of 90% in detecting advanced fibrosis with AUC = 0.791 (95% confidence interval: 0.649-0.933). At a cutoff value of 2.26 for FIB-4 index, the test had a sensitivity of 50% and a specificity of 87% in detecting advanced fibrosis with AUC = 0.797 (95% confidence interval: 0.683-0.911).

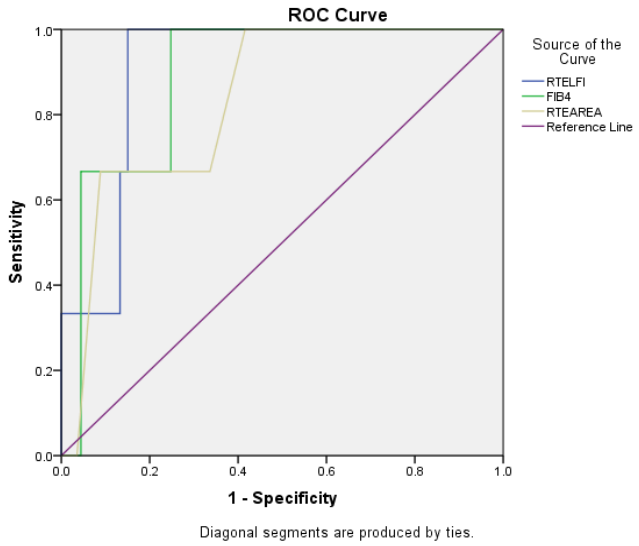

Figure (5): Receiver operating characteristic (ROC) curve for prediction of established cirrhosis (F4).

Figure (**5**) showed the ROC curve graphed to determine appropriate cutoff points of RTELFI and FIB4 score in predicting stage of liver fibrosis that give optimal sensitivity and specificity. The positive actual state is determining the cutoff value at which established cirrhosis (F4) could be determined. At a cutoff value of 3.3 for RTELFI, the test had a sensitivity of 100% and a specificity of 85% in detecting established cirrhosis with AUC= 0.906 (95% confidence interval: 0.817-0.994). At a cutoff value of 2.26 for FIB-4 index, the test had a sensitivity of 66% and a specificity of 83% in detecting established cirrhosis with AUC=0.888 (95% confidence interval: 0.769-1.000).

DISCUSSION

FIB-4 was validated in a large cohort of HCV mono-infected patients in whom values < 1.45 had a NPV of 94.7% to exclude severe fibrosis (F3-F4) with a sensitivity of 74.3%[9]. A FIB-4 value higher than 3.25 had a positive predictive value (PPV) of 82.1% with a specificity of 98.2%. The authors also showed a similar performance between FIB-4 and FibroTest®. Several other studies reported that the FIB-4 index had

a variable degree of accuracy in HCV-infected subjects[10].

Regarding the FIB-4, our results showed that there was a significant statistical relation between the degree of hepatic fibrosis and FIB-4 with p = 0.001. FIB-4 exhibited high reliability for predicting liver fibrosis in CHC patients, where higher values of FIB-4 were associated with more advanced stages of liver fibrosis. The mean FIB-4 index was significantly increased with the different stages of liver fibrosis assessed by liver biopsy: 1.24±0.45 at (F0); 1.38±1.17 at (F1); 1.63±0.93 at (F2); 2.50 ± 1.32 at (F3) and 3.58±1.51 at (F4).These results agreed with **Vallet-Pichard *et al.*[9], Holmberg *et al.*[11], Li *et al.*[12]** and **McCombs *et al.*** [13] who showed high diagnostic accuracy and utility of FIB-4 for assessing different stages of liver fibrosis.

Our results showed that FIB-4 appeared to be useful for evaluating patients for HCV advanced fibrosis (≥F3) with p value of0.002. This result is consistent with the results reported in the studies of **Khairy *et al.*[14]** and **Joo *et al.*[15].**

When we studied RTE LFI, our results showed a significant relation between the degree of hepatic fibrosis and RTE LFI (p <0.001). LFI measured by HI-RTE was an effective parameter for evaluating the degree of liver fibrosis. The LFI had been demonstrated to correlate well with the histological grade and was clinically applicable for the detection of liver fibrosis. The mean readings of RTE LFI significantly increased with the different stages of liver fibrosis assessed by liver biopsy: 1.48 ± 0.51 for F0, 2.48 ± 0.58 for F1, 2.75± 0.58 for F2, 3.38 ± 0.93 for F3 and 3.93 ± 0.92 for F4 (p <0.001).This agrees with the studies of **Koizumi *et al.*** [16] and **Kim *et al.*** [17] which concluded that RTE is useful for diagnosis of liver fibrosis regardless of stage in patients with chronic viral hepatitis.

In the current study, our results revealed that the LFI calculated by RTE showed a very good diagnostic performance to predict advanced fibrosis (≥F3 versus F0-F2) in CHC patients (p = 0.001). This was in concordance with **Lan *et al.*[18]** and **Marques *et al.*[19]** who suggested that LFI was excellent in diagnosing F≥3.

Our results showed a moderate positive binary correlation that was noticed between RTELFI and FIB-4 with Pearson Correlation "r" 0.391. These results agreed with **Kim *et al.*[17]** who concluded that as regards the prediction of advanced fibrosis, the combination formula of LFI and serologic parameters showed better AUROC than LFI or FIB-4 alone and combining serologic marker with LFI (multiplying LFI by FIB-4) improved the diagnostic performance.

In our study, a moderate positive binary correlation was noticed between RTELFI and fibrosis score with Pearson Correlation "r" 0.434. These results agrees with **Friedrich-Rust *et al.*** [20] and **Kim *et al.*[17]** where there was a strong positive correlation between the histologic liver fibrosis stage and the LFI. The mean LFI by RTE significantly increased with the histologic fibrosis stage.

We noticed a moderate positive binary correlation between FIB-4 and both necroinflammatory score and fibrosis score with Pearson Correlation "r" 0.382 and 0.396 respectively. This result agrees with **Amorim *et al.*[21]** and **Kitajima *et al.*[22]** where there were significant correlations between the stage of METAVIR fibrosis and FIB-4. Their results demonstrated that FIB-4 could be useful to assess fibrosis progression in LDLT recipients with HCV.

Receiver-operating characteristic (ROC) curves were constructed to determine appropriate cutoff points of RTE LFI and FIB4 score predicting stage of liver fibrosis that give optimal sensitivity and specificity. The positive actual state is determining the cut-off value at which advanced fibrosis (≥F3) could be determined. They both showed comparable diagnostic performance levels with AUC=0.797 versus AUC=0.791 for FIB4 and RTELFI respectively.

Regarding diagnosis of advanced fibrosis (≥F3), our study results revealed that at a cutoff value of 3.26 for RTE LFI, the test had a sensitivity of 72% and a specificity of 90% in detecting advanced fibrosis with AUC = 0.791. This is in agreement with **Ge *et al.*[23]** who reported that (LFI) at cut-off value of 2.5 for the diagnosis of fibrosis stage resulted in sensitivity of 65.5% and specificity of 87.9%. Our results disagrees with **Marques *et al.*[19]** where RTE showed a very good diagnostic performance for predicting advanced fibrosis (F≥3) in patients with CHC. Using the optimal cut-off value of 2.38, the AUROC for the LFI was 0.73 for predicting advanced fibrosis (F≥3) in chronic HCV, with excellent sensitivity 100% and specificity 65.2% but agreed with our results in RTE LFI that showed a very good diagnostic performance to predict advanced fibrosis (F≥3) in CHC, with similar results to FIB 4. In addition, disagrees also with **Mobarak *et al.*[24]** where RTE was able to diagnose significant hepatic fibrosis (F≥2) according to METAVIR scoring system at cut-off value of 2.49 with sensitivity 100%, specificity 66%, and AUROC 0.8. This high diagnostic performance is probably explained by exclusion of patients with BMI >30 from this study.

Regarding diagnosis of advanced fibrosis (≥ F3), our study results revealed that at a cut-off value of 2.26 for FIB-4 index, the test had a sensitivity of 50% and a specificity of 87% in detecting advanced fibrosis with AUC=0.797.Our results agrees with **Shah *et al.*[25]** and **Sumida *et al.*[26]**, where at FIB-4 cut-off value 2.67, the test had sensitivity of 52% and specificity of 90%with AUC of 0.8 for diagnosis of advanced Fibrosis. Our results disagrees with **Alboraie *et al.*[27]** where at FIB-4 cut off value 1.45 with AUC of 0.645, FIB-4 showed sensitivity 79% and specificity 77%. Also disagrees with **El Nakeeb *et al.*[28]**, where at FIB-4 cut off value 1.61, the test had sensitivity of 69.5% and specificity of 100% with AUC of 0.91 for diagnosis of significant fibrosis or cirrhosis (F3-F4). **Vallet-Pichard *et al.*[9]** concluded that FIB-4 index enabled the correct identification of patients with severe fibrosis (METAVIR F3-F4). FIB-4 index higher than 3.25 had a specificity of 98.2% and a sensitivity of 37.6% with AUC of 0.85 to confirm the existence of significant fibrosis (F3-F4).

Comparing between RTE-LFI and FIB-4 score for diagnosis of established cirrhosis (F4), our study revealed that RTE-LFI exhibited higher diagnostic accuracy than FIB-4 in diagnosis of established cirrhosis (F4) with more sensitivity and specificity. This comes in agreement with **Kim *et al.*[17]** and **Tamaki *et al.*[29]** where discriminating stage F3 and F4, only LFI had a significant power for predicting the stage F4, and LFI showed higher AUC compared to the FIB-4 index. RTE could discriminate between advanced fibrosis (F3) and cirrhosis. Our results disagree with **Tatsumi *et al.* [8]** where the cutoff value of RTE-LFI was 2.33 for F4 with sensitivity 48.5% and specificity 91.8%.

In our study, at a cutoff value (2.26) for FIB-4 index, the test had sensitivity of 66% and a specificity of 83% in detecting established cirrhosis with AUC =0.888.Our results disagree with **Yosry *et al.*[30]** and **Cordie *et al.*[31]**. The best cutoff levels for FIB4 for the prediction of advanced fibrosis 1.67 with sensitivity of 77% and specificity of 84%.Also, disagreed with **Taneja *et al.*[32]** where at FIB4 cutoff of 3.25, the sensitivity for diagnosis of cirrhosis was 57.9% and specificity was 95.7%.

CONCLUSION

Non-invasive methods, namely FIB-4 and RTE, had a good result in detecting degree of fibrosis. Moreover, besides that, RTE had excellent accuracy for F4 and F≥3 and it was superior to FIB-4.

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