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Can Combinations of Non-Invasive Parameters Replace Liver Biopsy in the Diagnosis of Hepatic Fibrosis in Egyptian Patients with Chronic Hepatitis C

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Authors' contributions

This work was carried out in collaboration between all authors. All authors shared in the study design and management of cases. Authors WAA, ME and NES performed the statistical analysis. Authors MHE, EMD and NAA wrote the first draft of the manuscript. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: Liver biopsy has always been represented as the standard reference for assessment of hepatic fibrosis although it has several limitations. This study aimed at evaluating the accuracy of noninvasive methods for diagnosis of hepatic fibrosis in adult Egyptian patients with chronic hepatitis C virus (HCV) infection.
Study Design: Cross sectional study.
Place and Duration of Study: This study was conducted in Al-Ahrar General Hospital (local treatment centre for Hepatitis C virus), Sharkia Governorate, Egypt and the Tropical Medicine Department, Zagazig University Hospitals, Sharkia Governorate, Egypt in the period from April 2011 to March 2012.
Methodology: Fifty chronic HCV patients were selected out of 255 chronic HCV patients awaiting assessment for combined pegylated interferon/ribavirin therapy according to the modified guidelines of the National Committee for Control and Prevention of viral Hepatitis C in Egypt. Diagnosis of HCV was confirmed by detection of anti-HCV antibody and positivity for HCV RNA for more than 6 months. All patients were assessed by liver biopsy and noninvasive methods namely aspartate transaminase/platelet ratio (APRI),

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abdominal ultrasonography measuring caudate/right lobe ratio and liver stiffness measurement.
Results: The accuracy in diagnosis of liver fibrosis using different methods in comparison to liver biopsy was 60%, 84%, 88%, 90%, 92% and 84% for APRI, ultrasonography, Fibroscan, combined Fibroscan/APRI, Fibroscan/ultrasonography and APRI/ultrasonography respectively. The sensitivity was 62.5%, 87.5%, 87.5%, 90.6%, 93.8 and 87.5 for APRI, ultrasonography, Fibroscan, combined Fibroscan/APRI, Fibroscan/ultrasonography and APRI/ultrasonography respectively. The specificity was 55.6%, 77.8%, 88.9%, 88.9%, 88.9 and 77.8 for APRI, ultrasonography, Fibroscan, combined Fibroscan/APRI, Fibroscan/ultrasonography and APRI/ultrasonography respectively.
Conclusion: Fibroscan appeared superior to APRI score and abdominal ultrasonography in diagnosis of liver fibrosis**.** Combined Fibroscan /ultrasonography performed better than other combinations for the prediction of significant hepatic fibrosis.

Keywords: Chronic hepatitis C; APRI; fibroscan; hepatic fibrosis; liver biopsy; ultrasonography.

1. INTRODUCTION

Chronic hepatitis C (HCV) is a global health problem that affects more than 170 million people worldwide [1], particularly in Egypt, where high prevalence rates were reported reaching up to 20% [2]. It is a progressive disease that can lead to cirrhosis, liver failure, hepatocellular carcinoma, and death [3]. Hence, it is very important that the disease be treated in its early stages, which will effectively reduce the associated morbidity and mortality [4]. Formation and accumulation of fibrosis in the liver is the common pathway that leads to evolutive liver disease. Precise staging of liver fibrosis is essential for patient management in clinical practice because the presence of bridging fibrosis represents a strong indication for antiviral therapy. Liver biopsy (LB) has always been represented as the standard of reference for assessment of this hepatic fibrosis [5]. Although biopsy is used to stage most cases of liver disease, it is well known that this procedure has several limitations [6], and hence development of non-invasive assessment methods to quantify hepatic fibrosis is justifiable. But, any new method needs to be compared with the standard LB [7]. The aim of this study was to compare accuracy of the non-invasive examination by aspartate transaminase/platelet ratio (APRI), abdominal ultrasonography measuring caudate/right lobe ratio and hepatic stiffness (Fibroscan) in comparison to the standard LB for assessment of hepatic fibrosis in adult Egyptian chronic HCV patients.

2. MATERIALS AND METHODS

2.1 Patients

This study was conducted in Al-Ahrar General Hospital (local treatment centre for Hepatitis C virus), Sharkia Governorate, Egypt and the Tropical Medicine Department, Zagazig University Hospitals, Sharkia Governorate, Egypt in the period from April 2011 to March 2012 and included 50 (with completed assessment parameters) chronic HCV patients with confirmed hepatic fibrosis by the liver biopsy. They were selected out of 255 (205 patients were excluded due to causes listed below) chronic HCV patients assessed for combined pegylated interferon/ribavirin therapy according to the modified guidelines of the National

Committee for Control and Prevention of viral Hepatitis "C" in Egypt. Diagnosis of HCV was confirmed by detection of anti-HCV antibody and positivity for HCV RNA for more than 6 months. They were 42 males and 8 females. Their mean age was 39.9±7.9 years.

2.2 Exclusion Criteria

Are those of the National Committee; exclusion of patients with other causes of liver disease rather than HCV (by laboratory and liver biopsy) including:

1- Co-infection with HBV.
2- Haemochromatosis.
3- Alpha –antitrypsin deficiency.
4- Wilson's disease.
5- Autoimmune disease (by ANA).
6- Alcoholic liver disease.
7- Obesity –induced liver disease.
8- Drug-induced liver disease.

Also patients with substance abuse (alcohol >80 gm/day, IV drugs and inhaled drugs) were excluded.

2.3 All the Patients were Subjected to:

1- Complete history taking,
2- Complete clinical examination
3- Routine laboratory investigations:

   a) Liver function tests.
   b) Prothrombin time and concentration and INR.
   c) Blood urea, serum creatinine.
   d) Complete blood counts.

4- Special investigation:

A) The APRI index [8]: was calculated as follow: aspartate transaminase (X upper limit of normal) X100/platelet count (10^9/L).

$$APRI = \frac{\text{AST level (/ULN)}}{\text{Platelet count } (10^9/L)} \text{ X } 100$$

B) Liver biopsy and quantification of liver fibrosis: Pathological examination was performed at the liver histopathology laboratory, Faculty of Medicine, Zagazig University. More than one pathologist revised the slides to minimize inter-observer variability that commonly affects interpretation of LB. Liver biopsies were paraffin-embedded and stained with haematoxylin-eosin and Masson trichrome stains, additional stains were used when needed. The biopsies were reviewed blindly without knowledge of any parameter. Hepatitis grading and staging were evaluated according to the METAVIR scoring system [9].

C) Upper abdominal ultrasonography: Abdominal ultrasonographic examination was done to estimate caudate lobe to right lobe ratio, diagnose liver cirrhosis, and measure the size of liver and spleen and to detect the presence of ascites, if any. Caudate/right lobe ratio is a simple parameter to calculate, with the idea is that with advancement of hepatic fibrosis there is atrophy of the hepatic right lobe and hypertrophy of the caudate lobe; consequently with more ratio (towards 1) the likelihood of cirrhosis is enhanced.

D) Liver elastography (Fibroscan): Liver elastography (LE) was measured by FibroScan (Echosens, Paris, France). Briefly, an ultrasound transducer probe is mounted on the axis of a vibrator. Vibrations of mild amplitude and low frequency are transmitted by the transducer, inducing an elastic shear wave that propagates through the underlying tissues. Pulse-echo ultrasound acquisitions are used to follow the propagation of the shear wave and to measure its velocity, which is directly related to tissue stiffness the elastic modulus: the stiffer the tissue, the faster the shear wave propagates. LE measures liver stiffness in a volume that approximates a cylinder of one cm wide and four cm long, between 25 mm and 65 mm below skin surface. This volume is at least 100 times bigger than a biopsy sample and is therefore more representative of the hepatic parenchyma. LE is painless, rapid (less than five minutes) and easy to perform in the outpatient clinic. The results were immediately available and were expressed in kilopascals (kPa), corresponding to the median value of 10 validated measurements and range from 2.5 to 75 kPa [10]. In this study fibrosis stages were calculated as follow: F0 (0-5 kPa), F1 (5.1-7 kPa), F2 (7.1-10 kPa), F3 (10.1-17.5 kPa), and F4 (17.5-75 kPa).

2.4 Ethical Considerations

The study was approved by the Ethical Committee of the Faculty of Medicine, Zagazig University. The patient gave a written consent for the procedures after explaining the risk/benefit ratio as well as expected hazards and interventions.

2.5 Statistical Analysis

Data were checked, entered and analyzed using SPSS version 15. Data were expressed as mean ± SD for quantitative variables, number and percentage for qualitative ones. Chi-squared (X^2) and t test were used when appropriate. $P < 0.05$ was considered significant. The most commonly used index of accuracy is the area under the receiver operator characteristic (ROC) curve (AUROC), with values close to 1.0 indicating high diagnostic accuracy. A patient was assessed as positive or negative according to whether the noninvasive marker (APRI, LE) value was greater than or less than a given cut-off value. Fibrosis was classified into mild (F1) and significant (F2-4). Comparison between different modalities to assess the sensitivity, specificity, positive and negative predictive values and AUROC were done.

3. RESULTS AND DISCUSSION

3.1 Base Line Characters

The base line characters for age, sex, body mass index (BMI), liver enzymes, platelets, APRI, Fibroscan and LB are shown in Table 1. The age range of this group lies within the age range of 18-60 years that is proposed by the national guidelines. Most of our patients were males (84%). None of our patients was morbidly obese (BMI≤33).

Table 1. Base line characters of the studied patients

	N = 50	
Age (years)		
$\bar{X}$ ± SD	39.9±7.9	
Range	23-56	
Gender	No	%
Female	42	84.0
Male	8	16.0
BMI (Kg/m^2)		
$\bar{X}$ ± SD	27.2±2.9	
Range	22-33	
ALT (IU/L)		
$\bar{X}$ ± SD	102.78±38.39	
Range	35-138	
AST(IU/L)		
$\bar{X}$ ± SD	64.78±39.25	
Range	29-127	
Platelets (10^3/ mm^3)		
$\bar{X}$ ± SD	165.0±46.89	
Range	111-251	
APRI		
$\bar{X}$ ± SD	0.97±0.72	
Range	0.251-3.95	
Fibroscan	No	%
F0	4	8.0
F1	16	32.0
F2	8	16.0
F3	16	32.0
F4	6	12.0
Liver Biopsy	N	%
F1	18	36.0
F2	22	44.0
F3	8	16.0
F4	2	4.0

3.2 APRI

At a cutoff 0.88 APRI can detect the significant hepatic fibrosis with sensitivity of 62.5%, specificity of 55.6%, positive predictive value of 71.4%, negative predictive value of 45.5% and accuracy of 60% (Table 2). Analysis of the AUROC was .712 (Fig. 1), with non-significant difference between mild (F1) and significant (F2-4) fibrosis.

3.3 Ultrasonographic Examination

Assessment of hepatic fibrosis by abdominal ultrasonography for assessment of caudate lobe to right lobe ratio showed sensitivity of 87.5%, specificity of 77.8%, positive predictive value of 87.5%, negative predictive value of 77.8% and accuracy of 84.0% (Table 2). Analysis of the AUROC was .809 (Fig. 2). Abdominal ultrasonography showed significant

difference between mild (F1) and significant (F2-4) fibrosis (P<0.001). These results have better accuracy patterns than APRI.

3.4 Fibroscan

Liver stiffness values by Fibroscan in our work ranged from 3.7 to 50.0 kPa. At a cutoff 7 kPa Fibroscan can detect the significant hepatic fibrosis with sensitivity of 87.5%, specificity of 88.9%, positive predictive value of 93.3%, negative predictive value of 80% and accuracy of 88% (Table 2). Analysis of the AUROC was .917 (Fig. 3).

Table 2. Validity of the non-invasive modalities in prediction of fibrosis as confirmed by biopsy

	Biopsy		Sensitivity	Specificity	Predictive value		Accuracy
	F2-4	F 1			Positive	negative	
APRI							
>0.88	20	8	62.5%	55.6%	71.4%	45.5%	60.0%
<0.88	12	10					
Kappa coefficient = 0.17±0.13				P = 0.1 (non-significant)			
Ultrasonography							
>0.5	28	4	87.5%	77.8%	87.5%	77.8%	84.0%
<0.5	4	14					
Kappa coefficient = 0.65±0.14				P<0.001** (highly significant)			
Fibroscan							
F2-4	28	2	87.5	88.9	93.3	80.0	88.0
F0-1	4	16					
Kappa coefficient = 0.74±0.14				P<0.001** (highly significant)			

These current data indicated that Fibroscan had good performance and it appeared superior to ultrasonography and APRI score in diagnosis of liver fibrosis.

In this work, there was discrepancy in the detection of liver fibrosis between Fibroscan and LB as Fibroscan detected 4 patients without fibrosis (F0), although all of them had fibrosis (F1) as diagnosed by LB. But all these patients had readings by Fibroscan more than 0 and less than 5 kPa.

Combination of Fibroscan and APRI in detection of hepatic fibrosis had sensitivity of 90.6%, specificity of 88.9%, positive predictive value of 93.5%, negative predictive value of 84.2% and accuracy of 90.0%. Combination of Fibroscan and ultrasonogtaphy in detection of hepatic fibrosis had the best results among all parameters in this study and showed sensitivity of 93.8%, specificity of 88.9%, positive predictive value of 93.8%, negative predictive value of 88.9% and accuracy of 92.0%. Combination of APRI and ultrasonography had the least prediction among the combinations of hepatic fibrosis and had sensitivity of 90.0%, specificity of 88.9%, positive predictive value of 93.5%, negative predictive value of 84.0% and accuracy of 90.0% (Table 3).

Table 3. Validity of combined parameters in prediction of fibrosis as confirmed by biopsy

Fibroscan/ APRI	Biopsy Positive	Biopsy Negative	Sensitivity	Specificity	Predictive Value Positive	Predictive Value Negative	Accuracy
Positive	29	2	90.6	88.9	93.5	84.2	90.0
Negative	3	16					
Kappa coeff. = 0.78±0.14				P=<.001** Highly significant			
Fibroscan/ ultrasonography							
Positive	32	2	93.8	88.9	93.8	88.9	92.0
Negative	2	16					
Kappa coeff. = 0.82±0.14				P<0.001** Highly significant			
APRI/ ultrasonography							
Positive	28	4	87.5	77.8	87.5	77.8	84.0
Negative	4	14					
Kappa coeff. = 0.69±0.14				P<0.001** Highly significant			

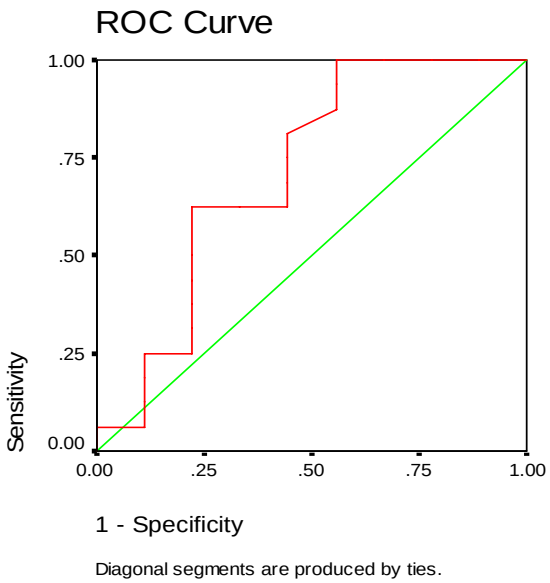

Fig. 1. AUROC for APRI

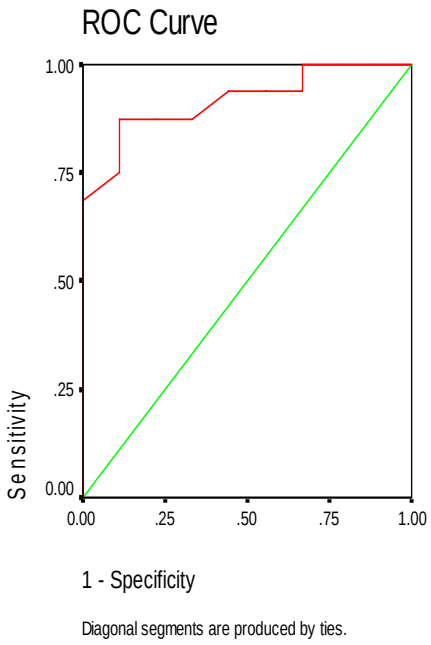

Fig. 2. AUROC for abdominal ultrasonography

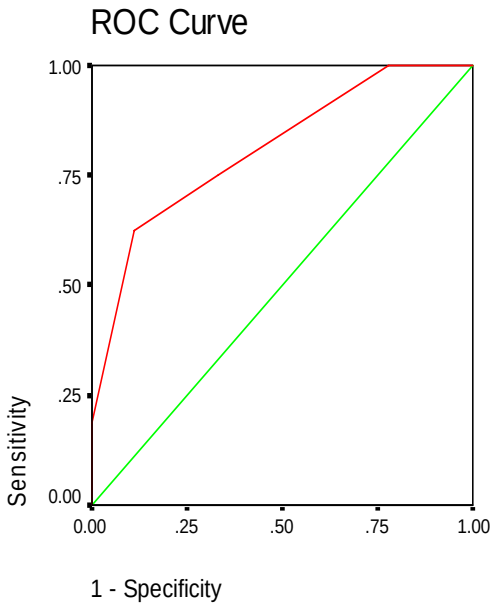

Fig. 3. AUROC for fibroscan

3.5 Discusion

Liver fibrosis is the main predictor of the progression of chronic liver diseases and is the main factor determining the prognosis and management [11], LB have long been used to assess liver fibrosis with many limitations and that is why non-invasive modalities are being developed.

In this work, the accuracy in diagnosis of liver fibrosis using different methods for detection was 60%, 84%, 88%, 90%, 92% and 84% for APRI, ultrasonography, Fibroscan, combined Fibroscan/APRI, Fibroscan/ultrasonography and APRI/ultrasonography respectively.

Liver biopsy is a costly procedure, difficult to be accepted by many patients, even many patients may discourage antiviral therapy due to their fear from LB [12], and it has several limitations. First, the biopsy procedure results in pain in 24.6% of patients [13]. Second, it has been shown that there is a high interobserver variation among pathologists for the staging of liver biopsy specimens [14]. Third, histological staging is based on a biopsy specimen that represents at most 1/50,000 of the total liver mass [12]. This, in addition to the fact that distribution of fibrosis in the liver parenchyma is heterogeneous, results in a no negligible sampling error resulting in up to 30% of false-negative results [6]. These limitations may lead to an underestimation of cirrhosis, especially when LB specimens are small or fragmented.

Ultrasonography imaging is used in clinical practice for the detection of advanced liver disease (mainly cirrhosis) either directly (by detecting overt morphological changes of the cirrhotic liver) or indirectly by detecting signs of portal hypertension (enlarged spleen, collateral vein circulation, etc.) because it is noninvasive, simple, rapid and readily available elsewhere [15]. Interestingly, ultrasonography evaluation of the liver surface was shown to be highly accurate for diagnosing clinically doubtful cirrhosis [16].

In the current study, we assessed the validity of ultrasonography in detection of fibrosis; it was found that ultrasonography had 87.5% sensitivity, 77.5% specificity and 84.0% accuracy in diagnosis of hepatic fibrosis mainly in significant fibrosis (F2-4). Aube et al. [17] reported that ultrasonography had accuracy of 82%-88% to 100% in assessing the diagnosis of cirrhosis. Its value is tempered by significant interobserver variability and an inability to gather all the required measurements, because of technical limitations [18]. However, ultrasonography can not quantify fibrosis and accurately diagnose it in absence of the stigmata of cirrhosis including shrunken liver and ascites which per se indicate cirrhosis and advanced fibrotic process and hence not accepted for assessment of hepatic fibrosis in patients waiting for combination therapy; instead it may be useful to exclude patients with advanced cirrhosis from antiviral therapy.

In this study, APRI was used because it is of low cost, easy to calculate and widely available almost everywhere [15]. By analyzing the diagnostic accuracy of APRI score for detection of fibrosis, it was found that at cutoff value of 0.88, APRI score had 62.2% sensitivity, 55.6% specificity and 60% diagnostic accuracy in detection of significant hepatic fibrosis in HCV patients. By analyzing the area under curve of APRI score, overall diagnostic accuracy of APRI was 0.712. This result indicated that APRI score had poor diagnostic validity in prediction of fibrosis. However, Castera et al. [19] reported that APRI showed similar performance to Fibroscan and Fibro test. Also, Wai et al. [8] compared the APRI and found areas under the ROC curves of 0.88 and 0.94 for the prediction of significant fibosis (Ishak fibrosis score of 3 and more) and cirrhosis (Ishak fibrosis score of 5 and 6) respectively. Although APRI was shown to discriminate HCV patients with and without cirrhosis [20], our

findings support the local Egyptian [21] and international opinions [22] about inability of APRI to correlate well with stages of hepatic fibrosis in patients with chronic HCV. This may be in one hand due to the questionable inter-laboratory reproducibility of some parameters such as transaminase levels and platelet count [15], and in the other hand to the prevalence of significant fibrosis in the population under study [23].

The main advantage of liver Fibroscan compared with fibrosis markers and biochemical scores is that it measures a quantitative physical parameter directly on the liver and there is no interference from extrahepatic disorders. It represents a totally different approach and therefore could be complementary of the fibrosis markers and biochemical scores to better assess liver fibrosis without using LB. Halfon et al., [24] and Rossi et al., [25] found that, the diagnostic performance of liver Fibroscan appears to be equivalent to that of the best biochemical scores for patients with significant fibrosis (F ≥ 2) and appears to be better than this test for the diagnosis of extensive fibrosis (F≥ 3) and cirrhosis (F = 4).

The limitations to liver Fibroscan included that Fibroscan cannot be applied in patients with ascites, even if clinically undetected. Ascites is a physical limitation to the technique because elastic waves do not propagate through liquids. However, the presence of ascites generally indicates by itself cirrhosis. In addition, liver Fibroscan is unsuccessful in patients with narrow intercostal spaces and in patients with morbid obesity [26]. None of our patients had ascites and this is because they were selected from patients awaiting assessment for pegylated interferon/ribavirin combination therapy. None of our patients was morbidly obese (all had BMI<33).

Assessment of the area under the curve showed that AUROC of Fibroscan for diagnosis of hepatic fibrosis was .917. At cutoff value of 7 kPa, Fibroscan had 87.5% sensitivity, 88.9% specificity and 88.0% accuracy. These current data indicated that Fibroscan had good performance and it appeared superior to ultrasonography and APRI score in diagnosis of significant liver fibrosis. This result agreed with that of Castera et al. [19] who reported Fibroscan as a noninvasive method for the assessment of liver fibrosis had diagnostic performance similar to that of methods based on serologic markers. Moreover, this result was in concordance with that of Carrie et al. [11] who reported that at cutoff value of 14.6 kPa, Fibroscan had 95% specificity, positive and negative predictive values for the diagnosis of cirrhosis were 74% and 96%, respectively, and the performance accuracy rate was optimal (92%).

Combination of Fibroscan with APRI improved the diagnostic accuracy in detection of hepatic fibrosis to 90%, a result better than each test alone but is lower than the results of the combined use of Fibroscan and ultrasonography which had the best diagnostic accuracy in this study with value of 92.0%, this result is better than combined APRI/ultrasonography which had accuracy of 84.0%. These findings are reinforced by other studies that used combination of Fibroscan with other biochemical markers to improve diagnostic accuracy for hepatic fibrosis, which may decrease the need for LB [27].

Our study had its limitations. First, the small number of patients recruited. Second, we did not perform Fibrotest as another non invasive method for detection of hepatic fibrosis to compare its diagnostic performance with that of APRI score and Fibroscan because of two reasons. Firstly, it is still a costly investigation in comparison to Fibroscan, ultrasonography and APRI at least in our community. Secondly, there were discrepancies of reports about its diagnostic validity. Gabrelli et al., [28] and Wong et al., [29] have investigated biochemical markers as alternatives to LB, these included fibrosis markers (procollagen III peptide, laminin,

hyaluronic acid), which are products of degradation or synthesis of extra cellular matrix. However, fibrosis is not specific to the liver. An impaired metabolism (renal failure, cholestasis) could influence blood levels of these markers. Moreover, they reflect dynamic processes such as fibro-genesis or fibrolysis rather than existent fibrosis.

4. CONCLUSION

In conclusion Fibroscan is easy, painless, rapid and simple method. It performs better than APRI and abdominal ultrasonography. Combination of Fibroscan and ultrasonography showed the best prediction parameters for diagnosis of hepatic fibrosis in Egyptian patients with chronic hepatitis C.

CONSENT

All patients gave a written informed consent for the procedures after explaining the risk/benefit ratio as well as expected hazards and interventions.

ETHICAL APPROVAL

The study was approved by the Ethical Committee of the Faculty of Medicine, Zagazig University.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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