Original Article

Predictors of Hepatic Fibrosis in Chronic Hepatitis C Virus Patients in Comparison to Liver Biopsy.

Nagwa I. Abdallah MBBCh[1], Ayman N. Menessy MD[1], Nancy A. Ahmed MD[1], Salah S. Arif MD[2]

Internal Medicine[1] and Clinical Pathology[2] Departments, Mansoura University, Egypt

Abstract:

Background/Aim: Liver fibrosis is the main predictor of the progression of chronic hepatitis C, and its assessment by liver biopsy can help the plan of therapy. However, biopsy is an invasive procedure with occasional complications and poor patient acceptance. The aim of this work was to compare non - invasive and invasive methods for evaluation of fibrosis in patients with chronic hepatitis C. **Methods:** This cross-sectional study was carried out at the Liver Unit of Mansoura University Hospital and Mansoura Health Insurance Hospital. The study was carried out on 100 patients with chronic active hepatitis, biochemical and virological studies were performed in addition to abdominal ultrasonography and liver biopsy in all patients, and also Serum fibronectin, APRI and AST/ALT ratio were performed. **Results:** We found that fibronectin has the highest sensitivity and specificity , the independent variables related to fibrosis were fibronectin, APRI, and AST/ALT ratio. **Conclusion:** The biochemical tests including APRI, AST/ALT ratio and particularly fibronectin could be valuable non – invasive predictor for assessment of liver fibrosis in chronic hepatitis C infected patients.

Keywords: H.C.V, fibronectin, Liver biopsy.

Introduction:

Hepatitis C Virus (HCV) infection is a major health problem in Egypt, where the seroprevalence is (10–20) fold higher than that in the USA, Egypt has the highest prevalence of HCV worldwide, ranging from 6% to more than 40% across regions and demographic groups. At present, liver biopsy is considered the 'gold standard' to evaluate the grade of liver fibrosis.(1) Liver fibrosis is the main predictor of the progression of chronic hepatitis C, and its assessment by liver biopsy (LB) can help the plane of therapy.(2) However, biopsy is an invasive procedure with occasional complications and poor patient acceptance.(3) Diagnosis of HCV infection is based on the presence of both anti- HCV antibodies, detected by enzyme immunoassays, and HCV RNA, detected by molecular assays. HCV RNA testing is essential for the management of HCV therapy.(4) Advanced liver fibrosis results in cirrhosis that can in turn lead to liver failure, portal hypertension and hepatocellular carcinoma. Fibrosis develops with different spatial patterns and is a consequence of various prevalent mechanisms according to the diverse causes of parenchymal damage. Early detection of fibrosis would allow for initiation of anti-fibrotic therapies capable of halting and even reversing this process.(5)

Pain is the most common complication of percutaneous liver biopsy, occurring in up to 84% of patients, including those with relatively mild discomfort.(6) The most important complication of liver biopsy is bleeding, which when severe occurs intraperitoneally.(7) A number of other complications have been reported after liver biopsy, these include pneumothorax, haemothorax, perforation of any of several viscous organs, bile peritonitis, infection, haemobilia and neuralgia.(8) Bellest et al., 2007 found that there is some contraindications in using biopsy; Absolute contraindications include: Severe coagulopathy, infection of the hepatic bed, extrahepatic biliary obstruction and Relative contraindications: Ascites, morbid obesity, possible vascular lesions, amyloidosis, hydatid disease, of similar importance to adequate specimen size is the necessity that a pathologist experienced in liver disease interpret the biopsy, ideally in partnership with the clinician

who performed the biopsy and /or whom is caring for the patient. In the absence of this interaction, diagnostic errors by non-specialist pathologists have been reported in more than 25% of patients evaluated at an academic centre.[9] Complex scoring systems, such as the Knodell scoring system and its revised form, the Ishak scoring system, have been devised for grading and staging of chronic viral hepatitis, as shown in the following table;[10]

IASL	Metavir	Batts-Ludwig
Grade(Activity, Inflammation) Minimal chronic hepatitis	A1	Grade1
Mild chronic hepatitis	A1	Grade2
Moderate chronic hepatitis	A2	Grade3
Severe chronic hepatitis	A3	Grade4
Stage (Fibrosis) Mild—Portal fibrosis	F1	Stage 1
Moderate—Periportal fibrosis or portal-portal septa	F1	Stage 2
Severe—Bridging fibrosis (few)	F2	Stage 3
Severe—Bridging fibrosis (many)	F3	Stage 3
Cirrhosis	F4	Stage 4

Several non-invasive tests have become available for clinicians to assess liver fibrosis and determine the best course of management for their patients, especially those with chronic hepatitis C.[11] Serum markers of liver fibrosis offer an attractive, cost effective alternative to liver biopsy for both patients and clinicians. In addition to being substantially less invasive, there are practically no complications, little or no sampling errors and small observer related variability. Moreover, measurements may be performed repeatedly, thus, allowing for a dynamic monitoring of fibrosis.[12] Fibronectin (FN) is a glycoprotein, produced from hepatocytes, Kupffer cells and endothelial cells ,Circulating fibronectin represents a viable marker for the presence of significant and advanced liver fibrosis in chronic hepatitis C patients (CHC) and fibronectin was identified at 90 kDa and quantified in sera of individuals with CHC using ELISA,[13] Fibronectin is one of the molecules produced by hepatic stellate cells, It is also part of the extracellular matrix, so it is important for the assembly of a collagen matrix in vitro , Its continuous presence also supports matrix integrity, both in vitro and in vivo ,It further regulates cell proliferation and cell cycle progression.[14] Fibronectin has been related to liver fibrosis and subsequent , development of portal hypertension in chronic liver disease.[15] Interpretation of serum aminotransferase levels, coagulation parameters, and platelet counts have been used in clinical practice to determine whether cirrhosis compensated or decompensated. Several studies have also evaluated the accuracy of combinations (or ratios) of these measures.[16]

Patients and methods

This study was carried out at the Liver Unit of Mansoura University Hospital and Mansoura Health Insurance Hospital. In the period between April 2012 and May 2014. The study was carried out on 100 Adult patients with chronic active hepatitis, of them 53 were males (53%) and 47were females (47%), informed written consent to participation into the study was obtained from each patient, inclusion criteria: age from 18 to 60 years, positive anti- HCV and HCV- RNA, patients with liver biopsy proven chronic hepatitis., HBs Ag negative and exclusion criteria: Age <18 and >60 years, co-infection with HBV, active alcohol consumption or features of alcoholic disease in the liver biopsy, HBs Ag

positive, preexisting psychiatric condition, pregnancy or breast feeding and co-morbidities.

All patients have been subjected to thorough history taking and complete clinical examination. Laboratory investigations included:- CBC (WBCs, hemoglobin & platelets). Bleeding time (BT), Alanine amino transferase (ALT) (U/L), Aspartate amino transferase (AST) (U/L), Serum bilirubin (mg/dl), Serum albumin (g /dl), Alkaline phosphatase (U/L), International normalized ratio (INR).

Viral markers were done including; HCV- antibody, HCV- RNA (IU/ m1) and Hepatitis B surface antigen, AST/ALT ratio (AAR), APRI (AST-to-Platelet ratio Index), ANA titre, SMA , AMA and LKM., TSH level, α feto protein (ng/dL), pregnancy test for females in child bearing period, Serum fibronectin (ng/ml).

Samples and standards were added and incubated in plates at 37 º C for 90 min. no wash ,Biotinylated antibodies were added and incubated in plates at 37 º C for 60 min. and washing 3 times with 0.01M TBS,ABC working solution was added and incubated in plates at 37 º C for 30 min. with washing 5 times with 0.01M TBS,TMB color developing agent were added and incubated in plates at 37 º C in dark for 20-25 min ,TMB stop solution were added and readed (BOSTER BIOLOGICAL TECHNOLOGY Co) and abdominal ultrasound.

Liver biopsy by highly qualified specialist in a well-equipped place under complete aseptic conditions, examined by a pathologist unaware of the laboratory results, needle liver biopsy specimens (n = 100) were taken from all patients. Biopsies were processed for diagnostic purposes, fixed in 10% neutral, buffered formalin, embedded in paraffin, cut into 4 µm thick, routinely stained with haematoxyline and eosin, the biopsies were pathologically classified according to Metavir staging system into different stages of fibrosis and cirrhosis from (F0-F4)

Statistical analysis:

Descriptive statistics were calculated for the anthropometric measurements and laboratory data in the form of: Mean ±Standard deviation (SD), Median and IQR, Frequency (No-%), using one of the following tests: - Student's *t*-test ANOVA Mann-Whitney *U*- test. The sensitivity and specificity of Fibronectin, APRI, AST/ALT ratio and serum albumin to diagnose fibrosis were examined at different cutoff points using ROC curve analysis to determine the best cut off point as well as the diagnostic power of each test. A *P* value <0.05 was considered statistically significant (S). And a *P* value <0.0001 was considered highly significant (HS) in all analyses.

Results:

Table (I) stages of fibrosis in studied cases according to Metavir:

Metavir	NO (n=100)	%
F0	12	12
F1	25	25
F2	28	28
F3	29	29
F4	6	6

Table (II) Serum albumin in different stages of fibrosis:

	Mean	SD	Median	IQR	P [a]
F0 (n=12)	4.29	0.26	4.40	4.03-4.50	**0.85**
F1 (n=25)	4.34	0.45	4.40	3.95-4.60	
F2 (n=28)	4.26	0.53	4.30	3.90-4.60	
F3 (n=29)	4.24	0.52	4.20	3.80-4.60	
F4 (n=6)	4.15	0.26	4.10	3.90-4.43	

***SD**: Standard deviation **IQR** (interquatile range)*

***P**: Probability a: ANOVA **p** value**<0.05** is significant.*

There is no significant difference in serum albumin level in the different stages of liver fibrosis.

Table (III) Serum Bilirubin in different stages of fibrosis:

	Mean	SD	Median	IQR	P value[a]
F0 (n=12)	0.96	0.34	0.85	0.63-1.28	<0.001
F1 (n=25)	1.01	0.44	0.90	0.70-1.20	
F2 (n=28)	1.14	0.29	1.20	0.88-1.38	
F3 (n=29)	1.27	0.39	1.30	0.90-1.60	
F4 (n=6)	1.88	0.26	1.90	1.65-2.13	

Serum bilirubin level is significantly increased with the progress of the degree of hepatic fibrosis.

Table (IV) Platelets counts in different stages of fibrosis:

	Mean	SD	Median	IQR	P value
F0 (n=12)	2.21E+11	3.11E+10	2.22E+11	1.92E+11-2.50E+11	0.01
F1 (n=25)	2.11E+11	4.88E+10	2.10E+11	1.77E+11-2.31E+11	
F2 (n=28)	2.09E+11	7.04E+10	1.84E+11	1.62E+11-2.40E+11	
F3 (n=29)	1.91E+11	5.57E+10	1.78E+11	1.53E+11-2.32E+11	
F4 (n=6)	1.54E+11	2.14E+10	1.57E+11	1.41E+11-1.68E+11	

E+10 *means* 10^{10}, **E+11** means 10^{11}

The increase in the degree of fibrosis is significantiy associated with decrease in platelet count.

Table (V) AST level in different stages of fibrosis:

	Mean	SD	Median	IQR	P
F0 (n=12)	19.75	15.06	15.50	6.25-35.00	<0.001[a]
F1 (n=25)	19.68	18.22	10.00	9.00-27.50	
F2 (n=28)	25.89	13.92	23.50	12.00-36.00	
F3 (n=29)	34.03	17.41	33.00	21.50-40.00	
F4 (n=6)	50.67	25.34	42.00	34.00-66.00	

AST is increased significantly with the increase in the stages of fibrosis.

Table (VI) ALT level in different stages of fibrosis:

	Mean	SD	Median	IQR	P value
F0 (n=12)	20.33	15.75	19.00	6.00-36.00	0.9
F1 (n=25)	21.19	19.95	12.00	6.00-38.00	
F2 (n=28)	20.82	16.96	14.00	7.50-36.00	
F3 (n=29)	21.31	16.92	15.00	8.50-36.00	
F4 (n=6)	25.00	24.45	14.50	4.75-56.00	

There are no significant changes in ALT level with the progress of the degree of liver fibrosis.

Table (VII) AST/ALT ratio in different stages of fibrosis:

	Mean	SD	Median	IQR	P value
F0 (n=12)	1.04	0.33	1.04	0.92-1.29	0.003
F1 (n=25)	1.13	0.62	0.96	0.76-1.18	
F2 (n=28)	1.89	1.70	1.12	0.85-2.59	
F3 (n=29)	2.65	3.13	1.65	0.97-3.42	
F4 (n=6)	4.07	3.18	2.91	1.54-7.59	

AST/ALT ratio is significantly increased with the increase in degree of fibrosis from (F0 – F4).

Table (VIII) APRI in different stages of fibrosis:

	Mean	SD	Median	IQR	P[a]
F0 (n=12)	0.14	0.13	0.09	0.05-0.19	<0.001
F1 (n=25)	0.20	0.16	0.15	0.10-0.24	
F2 (n=28)	0.30	0.17	0.26	0.15-0.40	
F3 (n=29)	0.36	0.22	0.30	0.16-0.54	
F4 (n=6)	0.62	0.55	0.40	0.28-1.00	

The progress of the stages of fibrosis from (F0 - F4) is associated with significant increase in APRI.

Table (IX) Serum fibronectin in different stages of fibrosis:

	Mean	SD	Median	IQR	P[a]
F0 (n=12)	18.83	14.33	15.00	8.25-31.50	**<0.001**
F1 (n=25)	36.64	25.77	26.00	15.00-53.50	
F2 (n=28)	58.46	37.38	52.50	22.50-86.75	
F3 (n=29)	93.00	83.51	70.00	25.00-115.00	
F4 (n=6)	152.50	94.11	135.00	71.25-232.50	

There is significant increase in serum level of fibronectin with the increase in stages of liver fibrosis from F0 to F4.

Table (X) sensitivity and specificity of different laboratory parameters in detection of fibrosis:

	Fibronectin	APRI	AST/ALT ratio	Serum Albumin
Area under the curve (AUC)	83.3 %	79.6%	61.7%	51.1%
Cutoff value	16	0.13	1.087	<4.25
Sensitivity	80.7	80.7	55.7	47.7
Specificity	77	77	42	58.3
PPV	94.6	94.6	90.7	89.4
NPV	32	32	15.2	13.2

PPV: Positive predictive value.
NPV: negative predictive value.

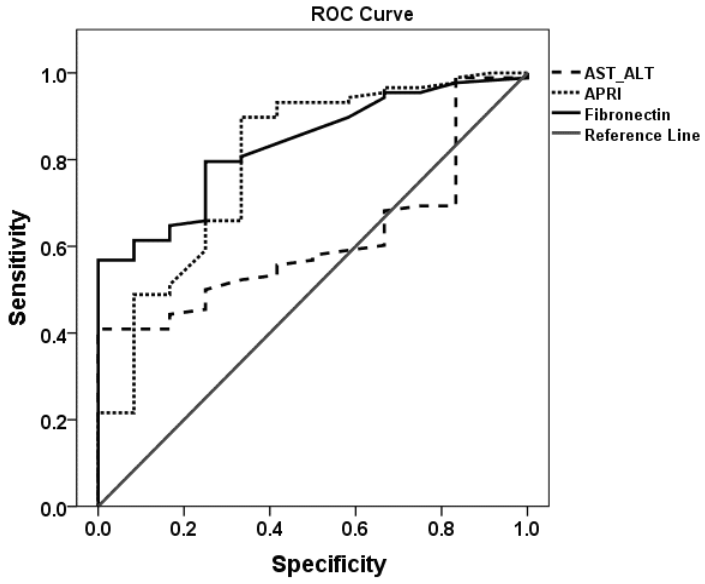

Figure (1): ROC Curve for Sensitivity & Specificity

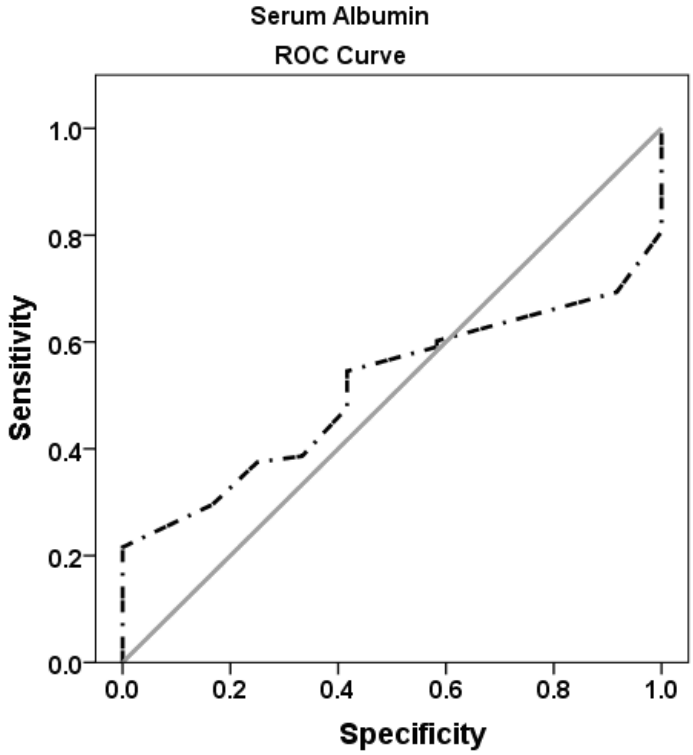

Figure (2): ROC Curve for Sensitivity & Albumin

As shown in **table X** and **figure (1**and **2)** above**,**

- ROC (receiver-operating characteristic) Curves of biomarkers for discriminating CHC patients **(F0)** from those with **(F1 - F4).**
- Using cutoff value (16, 0.13 ,1.087 and **<**4.25) the area under the curve (AUC) were 83.3, 79.6, 61.7 and 51.1 for fibronectin, APRI, AST/ALT ratio and albumin respectively.
- Increase sensitivity and specificity of fibronectin and APRI (80.7%and 77%) compared with AST/ALT ratio and albumin (55.7, 47.7, 42 and 58.3) respectively.
- PPV of fibronectin and APRI (94.6%) >AAR (90.7%) > albumin (89.4%).
- NPV of fibronectin and APRI (32%)> AAR (15.2%) > albumin (13.2%).

Discussion:

The gold standard for detecting liver fibrosis remains percutaneous liver biopsy, However, the procedure is limited by its invasive nature, expense, morbidity, intra- and inter-observer variability, and sampling errors,[17] Fibrosis prediction is an essential part of the assessment and management of patients with chronic liver disease. Blood-based biomarkers offer a number of advantages over the traditional standard of fibrosis assessment of liver biopsy, including safety, cost-savings and wide spread accessibility.[18] According to Metavir scoring system AST was increased (P value <0.001[a]) as shown in table (5). These results were in agreement with Ahmed et al,[19] who reported that AST was significantly increased with the progress of fibrosis stages. According to the progress in stages of fibrosis platelets count were decreased (P value =0.01[a]) as shown in table (4). Snyder et al. reported that low platelet count is caused by a variety of diseases as HCV.[20] In the current study the progress in the degree of fibrosis is associated with the increase in AAR (AST/ALT ratio) as

shown in table (7). Yu Hsieh et al. reported that the AAR scores increased significantly as the fibrosis advanced.[21] The level of the APRI was increased significantly with the progression of liver fibrosis stages as shown in table (8) ($P<0.001^a$).

Similar results were obtained by Ahmed et al. who reported that APRI was significantly increased with progression of fibrosis stages.[19] Similar results were obtained by Yilmaz et al., who reported that APRI was significantly associated with fibrosis scores in patients with CHC.[5]

In the current study Serum level of fibronectin was increased with the progress of the degree of fibrosis as shown in table (9) ($P<0.001^a$), similar results were obtained by Mosa et al.[15] In the current study the level of serum bilirubin was significantly increased within the progress of fibrosis stages ($P < 0.001$) as shown in table (3) ,This result was in agreement with Ahmed et al,[19] who reported that serum bilirubin was significantly increased with the progress in stages of fibrosis.

According to the stages of fibrosis (Metavir scoring system) the level of serum albumin was not significantly affected with the change in degrees of fibrosis ($P = 0.85$) as shown in table (2). Using ROC (receiver operating characteristic) curves to assess and compare the diagnostic accuracy of blood markers as fibronectin, APRI, AST/ALT ratio and serum albumin in patients with liver fibrosis ,In the current study as shown in table (10), and figure (1, 2), ROC curves of biomarkers for discriminating CHC patients with no liver fibrosis (F0) from those with liver fibrosis (F1 –F4). Using cutoff value (16, 0.13, 1.087 and <4.25) and the area under the curve (AUC) were 83.3, 79.6 , 61.7 and 51.1 for fibronectin, APRI, AST/ALT ratio and albumin respectively ,Sensitivity of fibronectin, APRI, AAR and albumin were(80.7%, 80.7%, 55.7% and 47.7%) respectively, Specificity of fibronectin, APRI, AAR and albumin were (77, 77, 42, and 58.3) respectively, PPV was (94.6, 94.6, 90.7 and 89.4) respectively for serum fibronectin, APRI, AAR and albumin, NPV was (32, 32, 15.2 and 13.2) for fibronectin, APRI, AAR and serum albumin respectively.

Conclusion:

Fibronectin has a better accuracy than APRI, AST/ALT ratio and albumin in prediction ability for fibrosis.

Recommendations:

In HCV patients because of its safety and cost effective. So, further studies aimed at reducing the need for liver biopsy.

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