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Prediction of Liver Fibrosis and Cirrhosis Among Egyptians Using Noninvasive Index

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Infection with hepatitis C virus (HCV) has become the most important public health problem in Egypt. Viral hepatitis and schistosomiasis are the major cause of chronic liver disease among Egyptians. Histological examination of the liver is an integral part of the evaluation of patients with chronic hepatitis C (CHC). Knowledge of the stage of liver fibrosis is essential for prognosis and decisions on antiviral treatment. Liver biopsy is currently the gold standard in assessing liver histology. Although percutaneous liver biopsy is in general a safe procedure, it is costly and does carry a small risk for complication. In addition; there could be sampling error because only 1/ 50,000 of the organ is sampled. Hence, there is a need to develop accurate and reliable noninvasive means to assess the severity of hepatic fibrosis. AST to platelets count ratio index (APRI) was developed to amplify the opposing effects of liver fibrosis and cirrhosis on AST and platelet count. This study was designed to predict significant fibrosis and cirrhosis using APRI-index using routine laboratory data among Egyptian patients with HCV. Using optimized cut-off values, significant fibrosis and cirrhosis could be predicted accurately in 71.4 % and 67.6 % respectively of 210 adult HCV Egyptian patients. Application of this APRI-Index may decrease the need for staging liver biopsy specimens among HCV patients.

Keywords: HCV, Schistosomiasis, APRI-Index.

Of all hepatitis viruses, only the hepatitis B virus (HBV) and hepatitis C virus (HCV) cause chronic hepatitis, which can progress to cirrhosis and hepatocellular carcinoma (HCC). HCV infection is estimated to affect 170 million people worldwide and constitutes a major public health problem. It causes a fluctuating chronic hepatitis that may progress to cirrhosis and hepatocellular carcinoma[1]. Although HCV infection is highly prevalent in Egypt, very little information is available on the distribution of the different genotypes of HCV. In most Egyptian patients, HCV genotype 4 is highly prevalent[2]. Liver biopsy is currently the gold standard in assessing liver histology. Although percutaneous liver biopsy is in general a safe procedure, it is costly and does carry a small risk for complications[3]. In addition, there could be sampling error because in only 1/ 50,000 of the organ is sampled. Furthermore, inter- and intra observer discrepancies of 10 % to 20% in assessing hepatic fibrosis have been reported, which may lead to understanding of cirrhosis[4]. Noninvasive approaches to assess histology among HCV patients may include clinical symptoms and signs, routine laboratory tests, serum markers of fibrosis and inflammation, quantitative assays of liver function, and radiologic imaging studies[5]. However, at present, none of these tests or markers alone are accurate or reliable in predicting histology. An ideal noninvasive diagnostic test for hepatic fibrosis should be simple, readily available, economic, and accurate[6]. An index comprising routinely available laboratory tests would meet these criteria. Many studies have been performed to

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evaluate the use of readily available laboratory tests results to predict significant fibrosis and cirrhosis among patients with chronic hepatitis[7]. For the prediction of cirrhosis, most studies examined the usefulness of predetermined formulae such as aspartate aminotransferase (AST)/ alanine aminotransferase (ALT) ratio or the cirrhosis discriminates score. Kaul *et al.*[8], performed univariate and multivariate analysis on 531 patients and derived a model consisting of gender, AST, platelets count, and spider nevi. This model was validated internally and externally with good accuracy but it included one subject variable.

MATERIALS AND METHODS

A. Patients

This retrospective cohort study included 210 consecutive adult patients with CHC from November 2001 to November 2003. The diagnosis of HCV was established by the presence of hepatitis C virus (HCV-RNA) using polymerase chain reaction assays. Patients with the following conditions were excluded from the study; presence of HCC, prior liver transplantation, prior interferon therapy, immunosuppressive therapy, insufficient liver tissue for staging of both fibrosis and cirrhosis and incomplete serological data.

B. Methods

1) The study included 210 adult patients under clinical investigation. Patients were classified according to the type of infection into five groups (Table 1).
2) Laboratory results performed within 4 months from the data of liver biopsy (CBC, complete liver function tests).
3) Results of serum aminotransferase (AST, ALT levels) were expressed as ratios of the upper limit of normal (ULN).
4) HCV-RNA level was expressed as Log_{10} IU/ml .
5) Abdominal ultrasound reports within 6 months from the time of biopsy were reviewed.
6) Histological slides from patients were retrieved, significant fibrosis was defined as Ishak score of 3 or more (presence of bridging fibrosis) and cirrhosis as Ishak score of 5 or 6.
7) Patients groups were studied for both significant fibrosis and cirrhosis according to two sets (training and validation sets), the obtained data were predicted for fibrosis and cirrhosis using APRI-Index

C. Statistical Analysis

Data were expressed as mean ±SEM unless otherwise stated. Statistical analysis was performed by Winks software version 4.65 Evaluation. There were 2 end points in this study, presence of significant fibrosis and cirrhosis. The variables were compared using both student's t and ANOVA test.

RESULTS

The results showed high prevalence for HCV infection (47.6 %), HBV (14.3 %), in relation to Bilharzia infection (11.9 %) which indicate the severity of these pathogens on public health as shown in Table 1. Approximately 25-30% of individuals with chronic HCV infections have persistently normal alanine aminotransferase (ALT) level and these individuals are usually referred to as healthy carriers of HCV. However, several studies have demonstrated that the histological features of most healthy carriers showed chronic liver damage of a variable degree, ranging from mild hepatitis to liver cirrhosis, and

Table 1. Clinical Classification of 210 patients included in this study.

Groups	Numbers(%)	PCR-Test	I.H.A. Test
HCV	100(47.62)	+ ve	- ve
HBV	30(14.30)	+ ve	- ve
HCV/HBV	30(14.30)	+ ve	- ve
Bilharzia	25(11.90)	- ve	+ ve
HCV/Bilharzia	25(11.90)	+ ve	+ ve

thus the existence of the true healthy carriers of HCV is still debatable. Because the relationships of serum ALT level to liver damage or viral replication in chronic HCV carriers remain unclear, liver biopsy is essential to evaluate the degree of liver damage in these subjects[9]. The severity of liver fibrosis was correlated significantly with a gradual increase in AST level as well as a decrease in platelet count however, there was significant overlap in AST and platelet among patients with different stages of fibrosis. To amplify the difference in AST and platelet values a novel index was devised called the AST to platelet ratio index (APRI):

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

APRI was correlated significantly with the stage of fibrosis, with a higher correlation coefficient than platelet, or AST level alone ($r = 0.60$. $p < 0.001$).

In training set

Two cut off points were chosen to predict the absence or presence of significant fibrosis (APRI ≤ 0.50) and (APRI > 1.5) respectively. The data obtained for patients groups were classified. The presence or absence of significant fibrosis or cirrhosis for each group is predicted as in Table 2; for example in HCV group, APRI of ≤ 0.50 was 6 patients of 100 (6%), would not have significant fibrosis. For patients with APRI of >0.5 & ≤ 1.5, (36 %) and (42 %) respectively, showed fibrosis. Patients with APRI value greater than 1.5 (58 %) were shown to have significant fibrosis. These results were matched with Ishak score for

Table 2. APRI Index in different liver diseases (HCV, HBV, HCV/HBV, Bilharzia, and HCV Bilharzia patients Groups) in training set model.

APRI index	Result of Prediction	HCV No=100 N (%)	HBV No= 30 N (%)	HCV/ HBV GroupNo = 30N (%)	Bilharzia No = 25N (%)	HCV /Bilharzia No= 25N (%)
Fibrosis						
≤ 0.50	Negative	6 (6)	3 (10)	N. D.	5 (20)	N. D.
> 0.50	Fibrosis	36 (36)	21 (70)	11 (37)	19 (76)	15 (60)
≤ 1.50	Fibrosis	42 (42)	24 (80)	11 (37)	24 (96)	17 (68)
> 1.50	Significant fibrosis	58 (58)	6 (20)	19 (64)	1 (4)	8 (32)
Cirrhosis						
≤ 1.0	Negative	26 (26)	24 (80)	4 (13)	24 (96)	6 (24)
> 1.0	Cirrhosis	35 (35)	5(16 .7)	14 (47)	3 (14)	14 (56)
≤ 2.00	Cirrhosis	39 (39)	30 (100)	20 (67)	25 (100)	20 (80)
> 2.00	Significant Cirrhosis	36 (36)	3 (10)	14 (47)	N.D.	5 (20)

*N>D.: not detected

Table 3. Correlation between the significant of APRI Index (mean ± SEM) in training and validation sets with type of infection.

Group	No.	APRI Mean ±SEM	Training Set		Validation Set	
			Fibrosis PPV (0.88)	Cirrhosis PPV (>2.00)	Fibrosis PPV (0.88)	Cirrhosis NPV (0.94)
HCV	100	2.0403 ±0.191	96 %	74 %	84 %	79 %
HBV	30	3.837± 2.836	90 %	20 %	36.7 %	33.3 %
HCV/HBV	30	2.488± 0.291	100 %	87 %	96.7 %	96 %
Bilharzia	25	0.720 ±0.0577	80 %	4 %	20 %	16 %
Bilharzia /HCV	25	1.537 ± 0.153	100 %	76 %	84 %	80 %

fibrosis. Also patients with APRI greater than 2.0 were shown to have significant cirrhosis Table 2. APRI values below cut off (0.50) and above cut off (1.5), 71.4 % of CHC patients could be identified correctly as either with or without significant fibrosis, also with cut off values of 1.0 and 2.0, the absence or presence of significant cirrhosis can be identified in 67.6 % of CHC patients (Table 3).

In validation set

Applying APRI to the validation set, two cut off for significant fibrosis and cirrhosis were chosen (0.88 & 0.94), respectively. Accuracy of using APRI for prediction in the validation set and its significant depends mainly on variables associated with type of infection as shown in Tables 4 and 5. For example in HCV groups the positive predictive value for fibrosis was 84 of 100 (84 %) of patients and 79 (79 %) for cirrhosis as shown in Table 6. The prediction of fibrosis and cirrhosis were highly significant especially in HCV, HCV/ HBV, and HCV/Billharzia groups.

Table 4. Univariate Analysis of Variables Associated with the presence of significant Fibrosis and Cirrhosis in different liver diseases.

Group	No	AST Mean ±SEM	ALT Mean ±SEM	AST/ULN Mean ±SEM	ALT/ULN Mean ±SEM	AST/ALT Ratio Mean ±SEM	PlateletCount (10^9/L) Mean ±SEM
HCV	100	131.44±16.9	100.2±9.852	2.398±0.10	2.027± 0.81	1.26± 0.096	143.82± 5.71
HBV	30	71.58 ±5.45	60.68±9.23	1.95 ± 0.12	1.35± 0.093	1.23± 0.085	202.56± 13.52
HCV/HBV	30	121.83±7.49	103.40±4.4	2.705±0.17	2.30±0.104	1.2±0.061	132±8.551
Bilharzia	25	78.45±3.034	71.611±4.4	1.740±0.07	1.636±0.11	1.154±0.049	266.12±15.20
Bilharzia / HCV	25	99.751±5.22	97.357±5.8	2.164±0.12	2.222±0.14	1.14±0.078	158.40±7.647

Table 5. Correlation between the Mean ± SEM of both, APRI Index, AST/ULN, and Platelet count with type of infection.

Group	No.	APRI Mean ±SEM	AST/ULN Mean ±SEM	Platelet Count (10^9 /L) Mean ±SEM
HCV	100	2.0403 ± 0.191	2.398± 0.097	143.82± 5.71
HBV	30	3.837± 2.836	1.59± 0.012	202.56±13.52
HCV/HBV	30	2.488± 0.291	2.705± 0.170	132± 8.551
Bilharzia	25	0.720 ± 0.0577	1.740± 0.066	266.12±15.198
Bilharzia HCV	25	1.537 ± 0.153	2.222 ± 0.139	158.40 ± 7.647

Table 6. Correlation between the Mean ± SEM of APRI Index and the prediction stage of hepatic fibrosis and cirrhosis with type of infection according to the positive and negative predictive values of APRI (0.88, 0.94) respectively in validation set.

Group	No.	APRI Mean ±SEM	Predicting of Liver Fibrosis PPV= (0.88)		Predicting of Liver Cirrhosis NPV= (0.94)	
			No of PPV (no.%)	No.of NPV (no.%)	No. of PPV (no.%)	No. of NPV (no.%)
HCV	100	2.0403 ± 0.191	84(84 %)	16(16%)	79(79 %)	21(21%)
HBV	30	3.837± 2.836	11(36.7%)	19(63.3%)	10(33.3%)	20(66.7%)
HCV/HBV	30	2.488± 0.291	29(96.7%)	1(3.3%)	29(96.7%)	1(3.3%)
Bilharzia	25	0.720 ± 0.0577	5(20%)	20(80%)	4(16%)	21(84%)
Bilharzia /HCV	25	1.537 ± 0.153	21 (84 %)	4(16 %)	20(80%)	5(20%)

DISCUSSION

Approximately 20 % of blood donors have been shown to be seropositive for HCV antibodies. The study suggests that the current high rates of HCV transmission may be due to unsafe injection practices used in a mass campaign to eradicate schistosomiasis in Egypt[10]. In this study, we attempted to use a single novel model comprising routinely available laboratory test results to predict significant fibrosis and cirrhosis in a consecutive series of treatment -naïve CHC Egyptian patients, the patients were grouped into five groups according to the type of infection as shown in Table 1. We found that platelet count, AST level and ALP level were the independent predictors for significant fibrosis, whereas platelet and white cell count, AST and ALP levels, as well as AST/ ALT ratio were independent predictors for cirrhosis. Our findings echoed results from many previous studies, which showed that platelet count, AST Level, and AST/ALT ratio were important predictors of either significant fibrosis or cirrhosis[11]. To amplify the opposite relationship between the stage of fibrosis and AST level and platelet count, Novel index, the APRI was used simply, its accuracy compared with models that comprise three or more variables in predicting of both fibrosis and cirrhosis. The performance of APRI in prediction was validated in a subsequent set of patients with similar accuracy. Many studies on prediction of significant fibrosis and cirrhosis among CHC patients have been published in the past few years[8]. This study showed several unique features, first, we recruited 210 consecutive CHC Egyptian patients under going liver treatment. Our study included treatment of naïve patients only because several studies have that liver histology may improve even among non responders to interferon-based therapy[12-13].

Secondly**,** this study included a sufficient proportion of patients with significant fibrosis (71.4 %) and cirrhosis (67.6 %), thus allowing us to study variables that could predict both of the study end points within the same patient population.

Although the overall study population only included 210 CHC Egyptian patients, and differences in race and mode of infection were present between the training and validation sets, the accuracy of APRI was validated in a sequential cohort of CHC patients undergoing a liver biopsy investigation. This suggests that the model is robust and accurate. Most importantly, the prediction model in this study consists of objective and readily available laboratory variables. Both platelet count and AST level are routine tests performed in CHC patients in clinical practice, so no additional tests are needed. The finding of decreased platelet count and increased AST level with progression of liver fibrosis has been reported in many studies. With increasing fibrosis and worsening portal hypertension, there is increased sequestration and destruction of platelets in the enlarging spleen[14]. In addition, studies in liver transplant patients showed that progression of liver fibrosis is associated with decreased production of thrombopoietin by hepatocyte and hence reduced platelet production[15-16]. Progression of liver fibrosis may reduce the clearance of AST, leading to increased serum AST level[17]. In addition, advanced liver disease may be associated with mitochondrial injury, resulting in more marked release of AST, which is present in mitochondria and cytoplasm, relative to ALT level[18-19]. The APRI was used in this study to amplify the difference in AST level and platelet count with different stages of fibrosis and cirrhosis. This novel index was accurate in predicting both significant fibrosis and cirrhosis and find the correlation between type of infection and severs of liver damages. The major advantage of the APRI is its simplicity. APRI can be determined in the clinic or bedside without the help of calculator. Moreover, the APRI allows clinicians to use a formula to predict significant fibrosis as well as cirrhosis. Finally, this study is based on the premise that liver biopsy is the gold standard for assessing hepatic fibrosis, but sampling error as well as intra- and interobserver variability can complicate the correlation between histology and noninvasive markers of hepatic fibrosis. In conclusion, we showed that a noninvasive, novel, simple index (APRI) consisting of two readily available laboratory results (AST level and Platelets Count), can predict significant fibrosis and cirrhosis among 210 Egyptian CHC patients with very high degree of accuracy. These results were validated in Egyptian patients with different modes of liver

infection, the result obtained using APRI clearly showed the relation between different blood borne pathogens (viral hepatitis and Bilharzia) and liver injury. This simple index may be of great importance in monitoring new trends of anti-viral as well as anti-fibrotic therapies.

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