Study of some Fibrosis Indices in Genotype 4 HCV Infected Egyptian Patients

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Abstract: Background and Aims: In HCV infected patients, liver biopsy is considered essential to stage liver fibrosis. Procedure of liver biopsy is invasive, expensive and not suitable for all patients. The present study aimed to evaluate the diagnostic accuracy of the readily available non-invasive fibrosis indexes for the fibrosis progression discrimination in chronic HCV mono infected and co-infected *Schistisoma mansoni* patients and to find a better combination of existing non invasive markers. **Methods:** The study included 100 genotype 4 HCV mono-infected and *S. mansoni* co-infected patients who underwent liver biopsy. The degree of fibrosis was scored according to the METAVIR staging system. The readily available AAR, APRI, FI, FCI, FT and FIB-4 serum indices. were tested in the patients. **Results:** There was a significant relationship between fibrosis stages and serum indexes except AAR and FCI ($P > 0.05$). AUROC of FT was higher than other indexes ($P < 0.05$) for differentiating minimal fibrosis (F1) from significant fibrosis (F2-F4). Also, FT showed high AUROC to predict cirrhosis. In HCV mono infected patients, minimal fibrosis can be identified using FCI and FT with sensitivity 57% for both , and specificity 58% and 57% respectively while cirrhosis can be identified using FI, FIB-4, APRI, FT, and AAR with sensitivity 100%, 75%, 100%, 50% and 100% and specificity 53%, 77%, 60%, 100%, and 59% respectively. In HCV/*S. mansoni* coinfection patients, minimal fibrosis can be identified using FT, FIB-4, APRI, FI, FCI and AAR with sensitivity 70%, 70%, 71%, 70% 60% and 60% and specificity 61%, 75%, 55%, 62%, 55% and 50% respectively while cirrhosis can be identified using FT, FI, FIB-4, and FCI with sensitivity 88%, 88%, 50% and 50% and specificity 52%, 70%, 60%, and 58% respectively. Moreover, *S. mansoni* anti-SEA was poorly significant with fibrosis stages. **Conclusion:** All methods used for predicting liver fibrosis were directly, and significantly, correlated with histological findings, but FT , FI, and APRI score had the strongest correlation with fibrosis severity while, AAR, and FCI showed significantly low 'r' index. These results suggest that the using FT as a first-line test in the social health centers seems feasible and effective.
[Niveen Saudy, Doaa M. Elghannam, Raghada Farag, Mohamed Abd El-Maksoud, Mona A. El-Hussiny, Mohamed El-Malky, Nader El-Malky. **Study of some Fibrosis Indices in Genotype 4 HCV Infected Egyptian Patients.** *Life Sci J* 2012;9(1):972-984] (ISSN:1097-8135). http://www.lifesciencesite.com. 141

Key words: HCV, *Schistisoma mansoni, fibrosis,* Egypt,

1. Introduction

Viral hepatitis C is a serious liver disease affecting 180 million people worldwide **(1).** The severity of the disease associated with Hepatitis C Virus (HCV) infection varies from asymptomatic chronic infection to cirrhosis and hepatocellular carcinoma **(2).** Egypt has the highest HCV prevalence in the world, with an overall prevalence of 12% among the general population, 40% in persons above 40 years of age, and even higher among persons in rural areas **(3).** Genotype 4 is the predominant genotype of HCV in Egyptian patients **(4).**

Schistosomiasis and HCV co-infection is common in Egypt. Some authors postulated an evidence of the association between the schistosomiasis treatment campaigns and the high HCV sero-prevalence rates observed in Egypt **(5).** Patients with HCV/*Schistosoma mansoni* co-infection have a more rapid progression of HCV liver fibrosis than do those with HCV infection alone and exhibited higher titers of HCV RNA **(6).** Schistosomiasis per se may cause the persistence of viremia due to reduced immunity **(7).** Prevalence of periportal thickening and fibrosis (PPT/F) increased significantly with increasing intensity of *S. mansoni* infection **(8)**.

Staging liver fibrosis is considered to be an essential part in the management of patients with chronic hepatitis C (CHC), because it provides prognostic information and, in many cases, assists in therapeutic decisions **(9)**. At present, liver biopsy is still most commonly used as reference standard for the assessment of liver fibrosis. However, its expense, risk of side-effects, and potential inaccuracy from sampling and observation errors reduce its utility for frequent liver fibrosis screening **(10).**

Currently, there are several non-invasive diagnostic methods for determining liver fibrosis that are being validated, such as blood markers and imaging methods **(11).** Several scoring systems like AST to ALT ratio (AAR), AST-Platelet ratio (APRI), Fibrotest (FT), Fibrosis Index (FI) and FIB-4 with different thresholds to predict presence or absence of fibrosis or cirrhosis in patients infected with HCV had been proposed **(12-17).**

Recently, a new marker FCI (fibrosis cirrhosis index) had been postulated to predict fibrosis in HCV infected patients **(18)**.

The purpose of our study was to evaluate and compare the diagnostic performance of the readily available non-invasive serum indexes including FT, AAR, APRI, FI, FIB-4 and FCI to find accurate and reliable non-invasive markers for evaluating fibrosis progression in HCV with or without Co schistosomiasis infection.

2. Methods

Patients

We carried out a retrospective cross-sectional study of all patients with documented HCV who underwent a liver biopsy between January 2010 and June 2011 at the outpatient clinic of the Tropical Medicine Department of Mansoura University Hospital. Elevated aminotransferase (ALT greater than 45 IU/L for >6 months (measured on at least two separate occasions), detectable levels of HCV-RNA and compatible hepatic histology were mandatory for the diagnosis of chronic liver disease secondary to HCV infection. Liver biopsies were performed on patients who were potential candidates for interferon plus ribavirin therapy. Consents were obtained from subjects included in the study. The study was approved by ethical committee of Mansoura Faculty of Medicine, Egypt.

Exclusion criteria

Patients who received a previous course of INF or immunosuppressive therapy or who had clinical evidence of Hepatitis B infection, HIV infection, end-stage renal disease, autoimmune disorders, liver cancer or complication of portal hypertension (variceal bleeding , encephalopathy, ascites, Child-Pugh B or C), were excluded from the study. Also, patients who had hemoglobin lower than 12 g/dl, pregnancy, neoplastic disease, uncontrolled psychiatric disease, severe cardiac disease; alcohol or drug abuse and a contraindication for liver biopsy (low platelet count < 70,000 plt/mm3, prolonged prothrombin time or decompensated liver cirrhosis) were excluded. This study included 100 patients (M/F 84/16; mean age 43.9 ± 7.8 (range 27-62 years).

HCV RNA detection and quantitative PCR

HCV infection was first documented in all patients by third-generation enzyme-linked immunosorbent assay (Abbott anti-HCV ELISA, Abbott Lab, IL, USA). RNA was extracted from 140 µl serum samples using QIAamp viral RNA extraction kit (Qiagen USA cat # 52906) according to the manufacturer's protocol. The HCV viral load was measured by Real time PCR, Stratagene Mx3000P Real-Time PCR System with a sensitivity of approximately 15 IU/ml.

HCV genotyping

Samples positive for HCV-RNA by real time PCR were subjected to genotyping of HCV, by RT-PCR for the core domain using the primers modified by **Ohno *et al.* (19).**

Histological evaluation of biopsy samples

The histological evaluation of paraffin-embedded liver specimens was carried out at the Pathology Department, Mansoura faculty of Medicine, following the recommendations of the Patient Care Committee of the American Gastroenterological Association **(20).** Ultrasound was routinely used to determine the percutaneous biopsy site. Liver fibrosis was estimated according to METAVIR scoring system **(21).** Histological staging based on the degree of fibrosis have five degrees of fibrosis: as F0 (no fibrosis), F1 (mild portal fibrosis without septa), F2 (moderate periportal fibrosis with few septa), F3 (severe fibrosis, fibrous septa with architectural distortion but with no obvious cirrhosis (bridging fibrosis) and F4 (cirrhosis).

We further grouped fibrosis stages as F0-F1 (minimal fibrosis), F2-F3 (advanced fibrosis), and F4 (cirrhosis), F2, F3, F4 (significant fibrosis).

Clinical and Laboratory data of Biomarkers of fibrosis

A complete clinical evaluation was performed on each patient. Immediately prior to the liver biopsy, 3 venous blood samples were obtained from all subjects and were processed in our hospital's laboratories'. EDTA blood samples were subjected to complete blood counts as (HB, Platelets, WBCs), by automated Sysmex 800. Sera were separated and tested for Albumin, aspartate aminotransferase (AST), ALT, total bilirubin, gamma-glutamyl transpeptidase (GGT), alkaline phosphatases (ALP) and total cholesterol ; using a Hitachi 902 Analyzer (Roche Diagnostics, Branchburg, NJ). Also, Prothrombin index, INR by Sysmex 540 coagulation analyzer (Dad Behring) was measured. Frozen serum stored at -80°C were analyzed for further assays to determine the special biomarkers designed to estimate the stage of fibrosis: Haptoglobin and Apolipoprotein A1 (Apo A1) concentration by Radial immunodiffusion (DIFFU-PLATE; Biocientifica®, SA, Buenos Aires), Alpha-2-macroglobulin: Quantitative determination using Turbidimitry technique (SPINREACT, S,A. Ctra, Santa Coloma)

Bilharisiasis ELISA

Serological detection of anti-*S masoni* IgG antibodies was done using the indirect ELISA technique where microtitration plates were sensitized using Schistosoma soluble egg antigen.

Anti-*Sm* IgG was tested by ELISA as follow;

Schistosoma mansoni soluble egg antigen (SEA) was prepared according to the method described **(22).** Total IgG responses to *S. mansoni* SEA was measured by indirect enzyme-linked immuo-sorbent assay

technique (ELISA) according to the general principles described by **Engvall and Perlmann (23)**. Briefly, Maxisorb polystyrene flat-bottomed micro-titration (Nunc, Roskilde, Denmark) plates were coated by overnight incubation (ON) at 4°C with 5 µg/ml antigen. The plates were washed six times in between each incubation step. Following the blocking step [0.1% (w/v) bovine serum albumin (BSA) (Fraction V, Sigma, MO, USA) in 0.035 M phosphate-buffered saline (PBS), pH 7.8, 1 h incubation at 37°C], the serum samples were loaded into the wells and incubated for 1 h at room temperature (RT). Sera were diluted 1/200 for IgG. All samples were tested in duplicate. As detecting antibodies, rabbit-anti-human IgG labeled with peroxides diluted 1/2000 (from Sigma, MO, USA) was used. Incubation times were 1 h at RT. Finally, the assays were developed using 3, 3', 5, 5' tetramethyl-benzidine (TMB) (Sigma, MO, USA), incubated for 15 min in the dark, and stopped by adding 20 % H2SO4. Absorbance at wavelength of 450 nm for substrate colour and 620 nm as reference was measured using ELISA Reader (Robbonic - India). Absorbance of the samples and the control plates without coating antigens were subtracted from the absorbance of the same samples. Antibodies concentration was calculated from optical densities.

The following scores were evaluated for predicting liver fibrosis: AAR, APRI (AST to platelet ratio index), Fibrosis Index (FI), Fibrotest (FT), Fibrosis / cirrhosis index (FCI) and FIB-4 indices.

- **AAR (12)**= AST (IU/l)/ ALT (IU/l)
- **APRI (13)**= [{AST (IU/l)/ ALT_ULN (IU/l)}×100]/ platelet count (10^9/l)
- **FI (15)** = 8.0 - 0.01 × PLT (109/l) - serum albumin(g/dl)
- **FIB-4 (16)** = [Age (Years) × AST (IU/l)]/[Platelet count (× 109/l) × ALT (IU/l)1/2]
- **FT (24)** = includes α2-macroglobulin, apolipoprotein A1, haptoglobin, total bilirubin, and GGT, adjusted for age and gender. Fibrotest was calculated using the following formula that is available on the USPTO website (http://www.uspto.gov; Patent no. 6,631,330): f = 4.467 log [a2-macroglobulin (g L)1)]) 1.357 log [haptoglobin (g L)1)] + 1.017 · log [c-glutamyl transpeptidase (IU - L)1)] + 0.0281 [age (years)] + 1.737 log [bilirubin (l mol L)1)]) 1.184 [apolipoprotein A1 (g L)1)] + 0.301 · sex (female = 0; male = 1)) 5.540 .
- **FCI (18)** = (ALP × Bilirubin) / (Albumin × Platelet count)

Statistical analysis

The data was analyzed using statistical package SPSS version 16 for windows. A *P* value of 0.05 was considered statistically significant. All data was presented as mean values. Spearman's rank correlation was used to assess the significant association between continuous variables and liver fibrosis stages. The student t-test was used to compare arithmetic means and parameters while Chi-square (X2) test was used to compare categorical data, correlation with Fisher's exact test was used when appropriate. Patients were divided into three main groups as, patients with no or minimal fibrosis (F1), patients with advanced fibrosis (F2-F3) and patients with cirrhosis (F4). The independently distinguished values of biochemical markers and AAR, APRI, FIB-4, FCI, FT and FI indices for the prediction of significant fibrosis and cirrhosis were evaluated using univariate regression analysis. Area under the receiver operating characteristic (ROC) curves (AUROCs) was used to compare and deduce the diagnostic accuracies of the selected biomarkers.

3. Results

The demographic and clinical outcomes of the 100 HCV genotype 4 infected patients explained in **Table 1**. The evaluation of chronic HCV activity (inflammatory grade) showed mild chronic hepatitis in 50 patients, moderate chronic hepatitis in 30 patients and severe chronic hepatitis in 20 patients. According to the Metavir scoring system, the severity of liver fibrosis in the study group of 100 patients with chronic hepatitis C was graded as follows: 50 patients had stage 1 fibrosis (F1); 29 patients had stage 2 fibrosis (F2); 9 patients had stage 3 fibrosis (F3); and 12 patients had cirrhosis (F4).

The different variables in fibrosis stages **(Table 2).** Most of studied patients were of mild and moderate fibrosis (F1-F2) (79/ 100). The distribution of liver fibrosis stages with regard to age and gender of patients showed no significant differences. Viral load was significant among fibrosis stages. It gradually increased in advanced fibrosis and cirrhosis. The discriminative values of the biochemical markers for the prediction of different fibrosis stages were determined by logistic regression analysis. By univariate analysis ($P< 0.05$), viral load, Hb level, bilirubin, ALT, AST, platelet count and haptoglobin levels were significantly associated with various fibrosis stages.

Schistosma mansoni (*S. mansoni*) Soluble Egg antigen antibodies (anti-SEA) levels were significantly different between fibrotic stages with higher levels in cirrhotic patients

The relationship between the fibrosis stages and six serum indices: AAR, APRI, FI, FT, FCI and FIB-4 is illustrated in **Figure 1&Table 2.** There was a significant relationship between fibrosis stages and serum indexes ($P < 0.05$) except AAR and FCI. A gradual increase in the level of APRI, FI, FT and FIB-4 indexes was observed in fibrosis stages. The cutoff

values and AUROCs of the serum non-invasive indices scores are shown in **Table 3**.

In HCV patients (both *S. mansoni* positive and negative), we analyzed the sensitivity and specificity of each index for minimal (F1), advanced (F2-F3), and cirrhosis (F4). In HCV/*S. mansoni* coinfection patients, minimal fibrosis can be identified using FT, FIB-4, APRI, FI, FCI and AAR with sensitivity 70%, 70%, 71%, 70% 60% and 60% and specificity 61%, 75%, 55%, 62%, 55% and 50% respectively while cirrhosis can be identified using FT, FI, FIB-4, and FCI with sensitivity 88%, 88%, 50% and 50% and specificity 52%, 70%, 60%, and 58% respectively

In HCV mono infected patients, minimal fibrosis can be identified using FCI and FT with sensitivity 57% for both , and specificity 58% and 57% respectively while cirrhosis can be identified using FI, FIB-4, APRI, FT, and AAR with sensitivity 100%, 75%, 100%, 50% and 100% and specificity 53%, 77%, 60%, 100%, and 59% respectively.

AUROC of FT was higher than other indexes ($P < 0.05$) for differentiating minimal fibrosis (F1) from significant fibrosis (F2-F4) **(Figure 2).** As shown, the order of performances of blood tests for minimal or significant fibrosis were differed from that of cirrhosis.

Spearman correlation between each serum index score and fibrosis stages was high for FT, FI, and APRI **Table 4**.

All methods used for predicting liver fibrosis were directly, and significantly, correlated with histological findings, but FT (r = 0.62), FI (r = 0.57), and APRI score (r = 0.51) had the strongest correlation with fibrosis severity while FFIB-4, AAR, and FCI showed significantly low 'r' index**.** Moreover, *S. mansoni* anti-SEA was poorly significant correlated with fibrosis stages.

The mean values of serum indices for minimal fibrotic HCV patients were illustrated in **Tables 5.** The most reliable indices were FT, FI, FIB-4 and APRI for distinguishing between different fibrotic stages in HCV/*S. mansoni* coinfection patients. As regard HCV mono infected patients.

Table 1: Demographic, clinical, and liver histological features of 100 chronic HCV genotype 4 infected patients.

Features	Patients Mean (± SD)
Sex (Male/Female)	84/16
Age (years)	43.9 ± 7.8
Viral load (IU/ml)	$1.46\times10^6 \pm 3\times 10^6$
Hb level (12-16g/dl)	13.6 ± 1.46
Platelet count (140-450 $\times10^9$/l)	170±46
ALT (0-45IU/l)	64.2±39.2
ALP (0-92IU/l)	60.67±26.1
AST (0-40IU/l)	63.8±45.7
GGT (0-40IU/l)	47.1±19.7
Total Bilirubin (0.1-1mg/dl)	1.24±0.3.7
Albumin (3.5-5.1 g/dl)	4.1±0.47
Apolipoprotein A1 (0.7-1.69 g/L)	1.7±0.82
Haptoglobin, (0.8-3 g/L)	0.96±0.69
Alfa2-Macroglobulin (1.3-3 g/L)	2.94±0.96
B eliza Positive (OD≥0.4)	0.6327±0.41
AAR	1.05±0.46
FT	0.42±1.41
APRI	0.68±0.6
FI	1.98±0.78
FIB-4	0.59±0.57
FCI	0.38±0.26
Histological Fibrosis stage	
F1(minimal fibrosis)	50
F2	29
F3	9
F2+F3 (advanced fibrosis)	38
F4 (Cirrhosis)	12
F2+F3+F4 (Significant fibrosis)	50

Table 2: The different variables in fibrosis stages.

Features	F1 (n =50)	F2 (n =29)	F3 (n = 9)	F4 (n =12)	*P* value
Sex (Male/Female)	43/7	23/6	9/0	9/3	0.425
Age (years)	42.5±5	45.2±5.4	45.7±9.3	50.2±11.6	0.394
Viral load ($\times10^6$ IU/ml)	0.76±0.87	1.72±3.39	0.35±0.66	4.49±5.92	0.005
Hb level (12-16g/dl)	14.6±1.4	13.9±1.6	13.1±1.2	12.8±0.8	0.008
Platelet count (140-450 $\times10^9$/l)	203±46.2	169±37.1	146±25.3	125±57.3	0.004
ALT (0-45IU/l)	61.7±44	61.3±38	60.1±21	84.1±24	0.018
ALP (0-92IU/l)	57.4±18.8	59.7±30.5	70.8±48.8	70±19.1	0.215
AST (0-40IU/l)	62.1±56	58.3±35	68.7±24	80.7±16	0.005
GGT (0-40IU/l)	45±18.9	47±20	50±21.2	50.4±23.5	0.68
Total Bilirubin (0.1-1mg/dl)	0.9±0.28	1.2±0.43	1.4±0.13	1.5±0.3	0.000
Albumin (3.5-5.1 g/dl)	4.2±0.48	4.3±0.49	4±44	3.8±0.24	0.082

Apolipoprotein A1(0.7-1.69 g/L),,	1.8±0.76	1.7±0.77	1.3±0.29	1.6±0.54	0.251
Haptoglobin (0.8-3 g/L)	1.23±0.76	0.81±0.51	0.72±0.38	0.84±0.37	0.000
a2-macroglobulin (1.3-3 g/L)	2.83±0.88*	2.91±1.2	2.95±0.89	3.41±0.63*	0.276 (0.039*)
B Eliza Positive (79/100) (OD≥0.4)	43/50 0.41±0.9	23/29 0.45±0.5	5/9 0.66±0.1	8/12 0. 81±0.2	0.002
AAR	1.05±0.49	1.03±0.51	1.15±0.77	1.02±0.33	0.935
FT	0.37±1.2	0.4±1.57	0.53±1.0	0.51±0.86	0.001
APRI	0.58±0.4	0.64±0.33	1.44±1.07	0.65±0.25	0.011
FI	1.81±0.83	1.92±0.73	2.6±0.77	2.38±0.34	0.013
FIB-4	0.49±0.35	0.58±0.32	1.18±1.64	0.63±0.31	0.015
FCI	0.34±0.24	0.45±0.31	0.41±0.39	0.39±0.28	0.315

Table 3: Performance indices of serum AAR, APRI, FIB-4, FCI, FT and FI in 100 chronic HCV genotype 4 infected patients.

		Minimal fibrosis (F1)				Advanced fibrosis (F2-F3)				Cirrhosis (F4)			
		cutoff	Sen %	Spe%	AUC [95% CI]	cutoff	Sen%	Spe%	AUC [95% CI]	cutoff	Sen%	Spe%	AUC [95% CI]
AAR	Total	1	57	48	0.523	1.01	65	68	0.59	1.06	50	63	0.489
	Neg B	1	50	43	0.337	1.05	100	60	0.588	1.02	100	59	0.588
	Pos B	1	60	50	0.591	1.07	58	70	0.574	0.81	75	35	0.400
APRI	Total	0.56	62	68	0.633	0.598	70	68	0.675	0.588	50	61	0.580
	Neg B	0.58	50	43	0.398	0.574	100	61	0.721	0.58	100	60	0.721
	Pos B	0.46	71	55	0.714	0.64	63	71	0.663	0.44	100	40	0.473
FIB-4	Total	0.48	62	66	0.649	0.500	70	64	0.677	0.471	60	51	0.607
	Neg B	0.751	43	85	0.388	0.59	75	78	0.735	0.75	75	77	0.735
	Pos B	0.479	70	75	0.725	0.48	75	62	0.674	0.51	50	60	0.541
FI	Total	2.13	59	64	0.614	2.29	85	62	0.706	2.39	83	66	0.679
	Neg B	1.86	43	32	0.367	2.35	75	89	0.794	1.87	100	53	0.794
	Pos B	2.125	70	62	0.67	2.29	88	65	0.682	2.36	88	70	0.621
FT	Total	0.409	74	54	0.73	0.460	80	62	0.76	0.462	75	58	0.702
	Neg B	0.478	57	57	0.53	0.57	50	100	0.66	0.58	50	100	0.66
	Pos B	0.420	70	61	0.74	0.42	88	57	0.77	0.42	88	52	0.70
FCI	Total	0.29	57	52	0.603	0.29	55	50	0.549	0.32	50	55	0.508
	Neg B	0.31	57	58	0.536	0.33	50	65	0.471	0.37	50	65	0.471
	Pos B	0.28	60	55	0.604	0.28	63	50	0.576	0.35	50	58	0.510

Table 4: Spearmanrank **Correlation between different tests and liver fibrosis (assessed according Metavir score).**

Test	Spearman's rank correlation coefficient	*P* value
FT	0.621	0.001
FI	0.57	0.007
FCI	0.32	0.04
AAR	0.29	0.043
APRI	0.51	0.000
FIB-4	0.47	0.005
Bilh antibodies	0.342	0.001

Table 5: Mean of values of six indices in minimal fibrotic HCV genotype 4 patients.

	Negative (21)	Mean	*p*	Positive (79)	mean	*p*
Fibrotest F1 (50) F2-F4(50)	 7 14	 0.45 0.48	 0.856	 43 36	 0.361 0.47	 0.000
AAR F1 (50) F2-F4(50)	 7 14	 1.30 1.03	 0.255	 43 36	 1.01 1.06	 0.167
FCI F1 (50) F2-F4(50)	 7 14	 0.33 0.4	 0.799	 43 36	 0.34 0.42	 0.116
APRI F1 (50) F2-F4(50)	 7 14	 0.795 0.650	 0.488	 43 36	 0.54 0.82	 0.001
FIB-4 F1 (50) F2-F4(50)	 7 14	 0.687 0.632	 0.443	 43 36	 0.46 0.72	 0.001
FI F1 (50) F2-F4(50)	 7 14	 2.28 1.94	 0.360	 43 36	 1.73 2.23	 0.012

Fibrotest

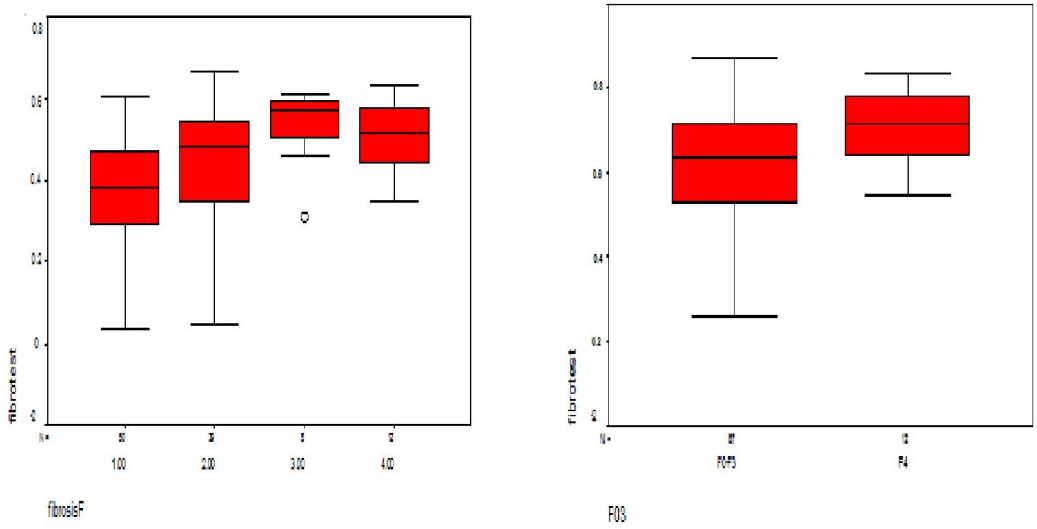
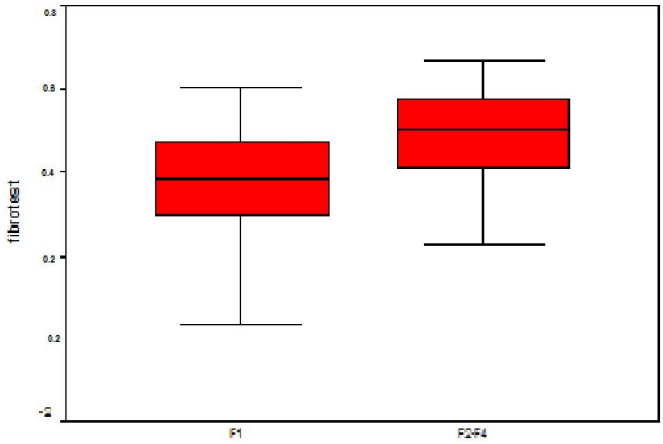
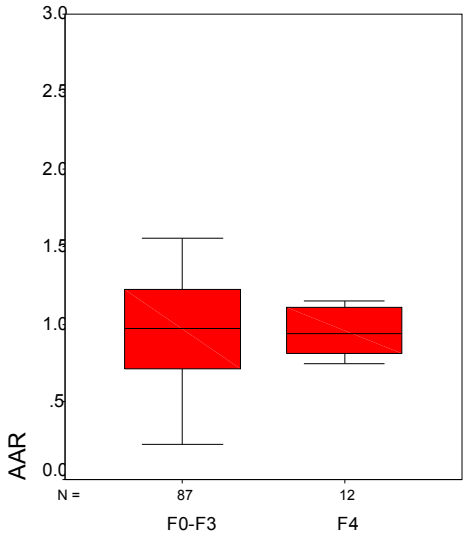


Figure 1 Box plots of the FT, FCI, AAR, APRI, FIB-4 and FI for different fibrosis stages. The horizontal line inside each box represents the median, while the top and bottom of boxes represent the 25th and 75th percentiles, respectively. Vertical lines from the ends of the box encompass the extreme data points.



AAR **APRI**



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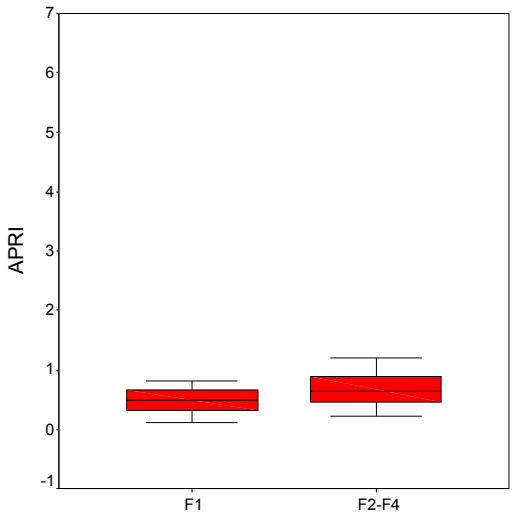
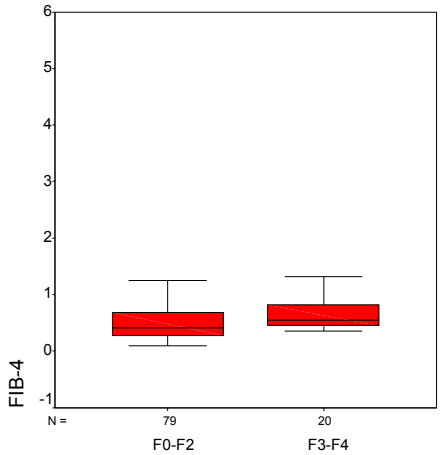


FIB-4



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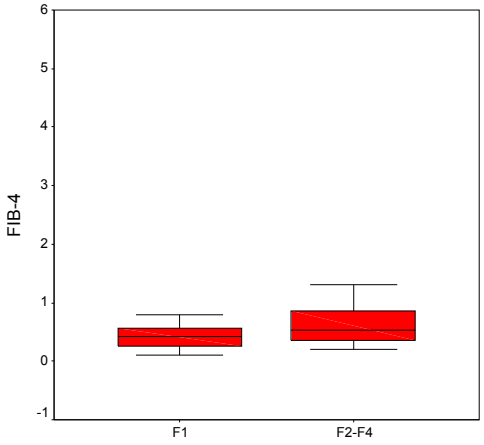
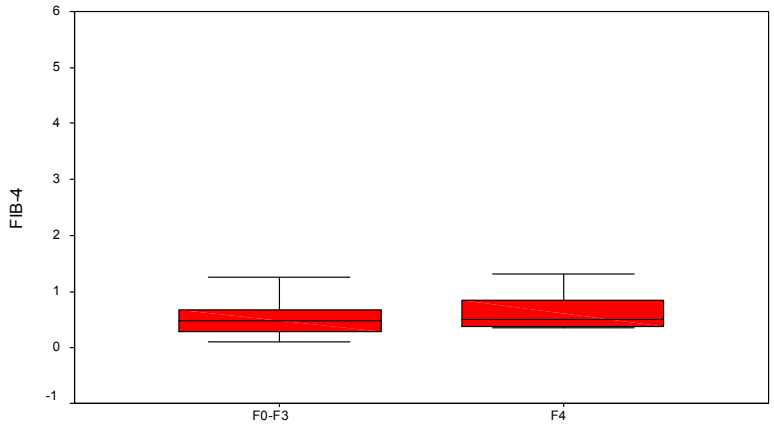
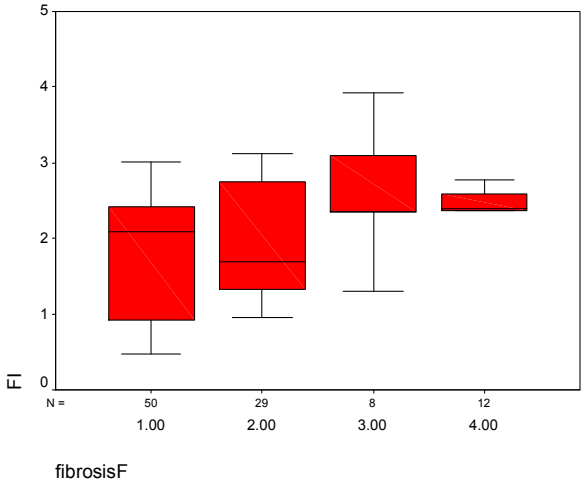




FI



FCI

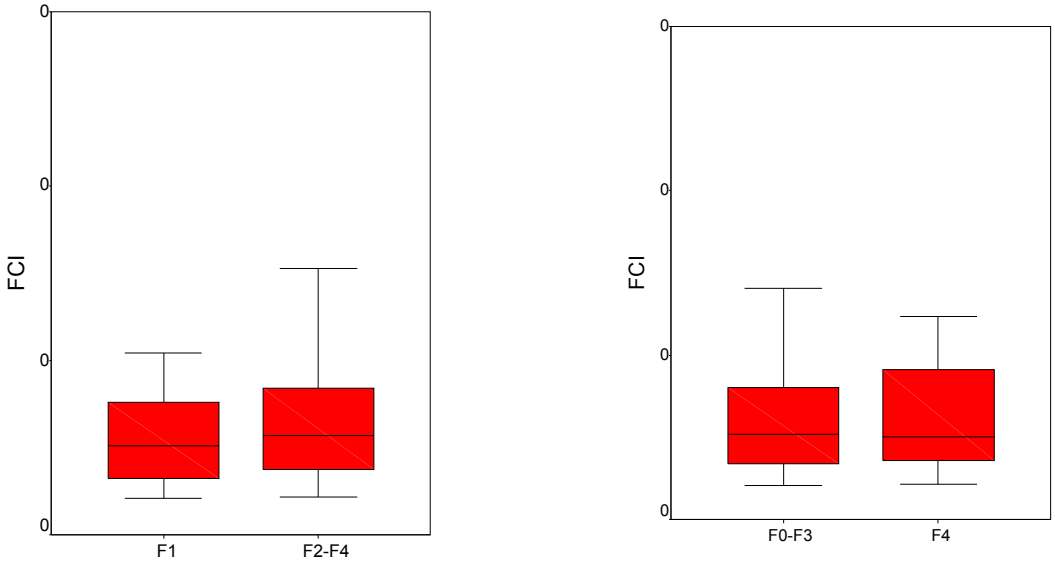
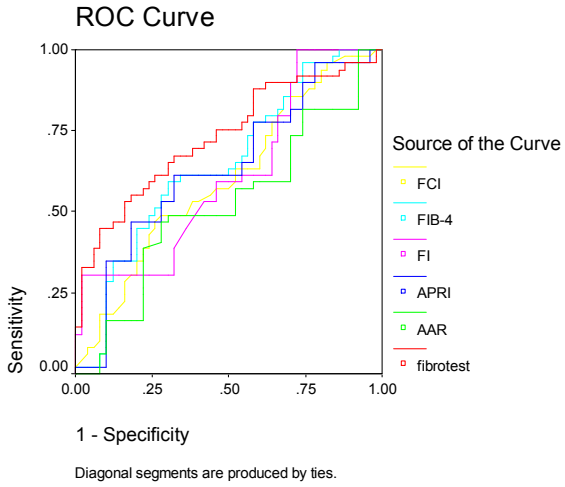


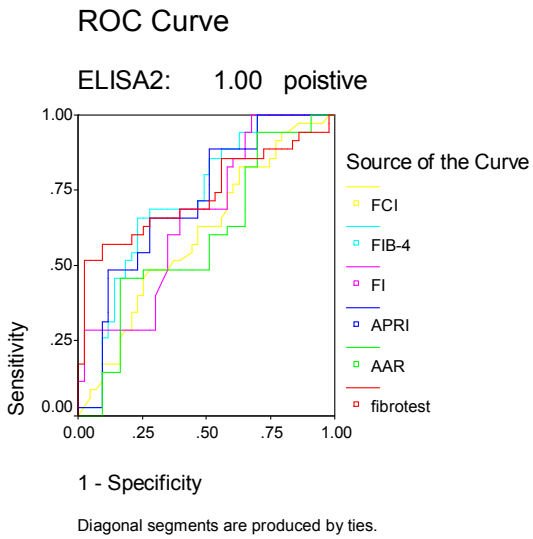
Figure 2: Receiver operating characteristic curves generated by six serum markers, AAR, APRI, FIB-4, FCI, FT and FI for differentiation between patients in fibrosis stage F1, F2-F3 and F4.

F1



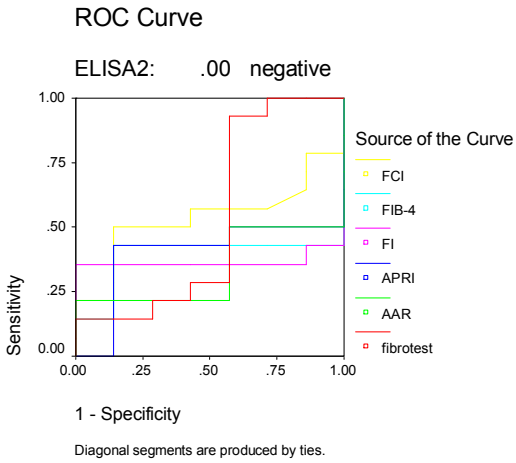
Area Under the Curve

Test Result Var	Area
fibrotest	.731
AAR	.523
APRI	.633
FI	.614
FIB-4	.649
FCI	.603



Area Under the Curve [a]

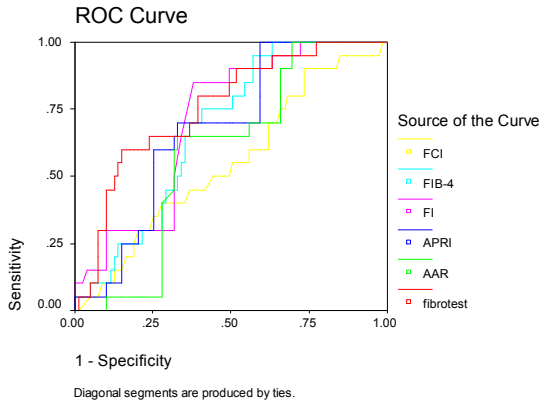
Test Result Variable(s)	Area
fibrotest	.736
AAR	.591
APRI	.714
FI	.665
FIB-4	.725
FCI	.604



Area Under the Curve[a]

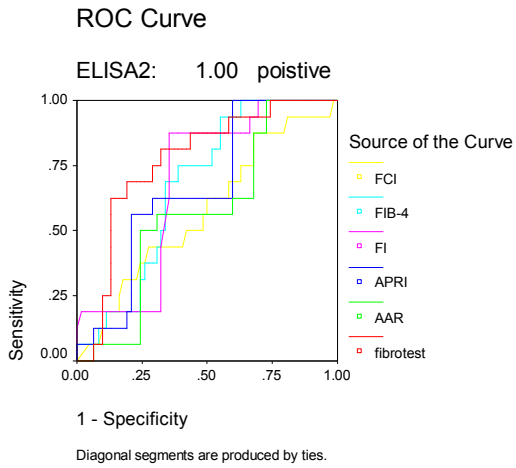
Test Result Variable(s)	Area
fibrotest	.531
AAR	.337
APRI	.398
FI	.367
FIB-4	.388
FCI	.536

F2-F3



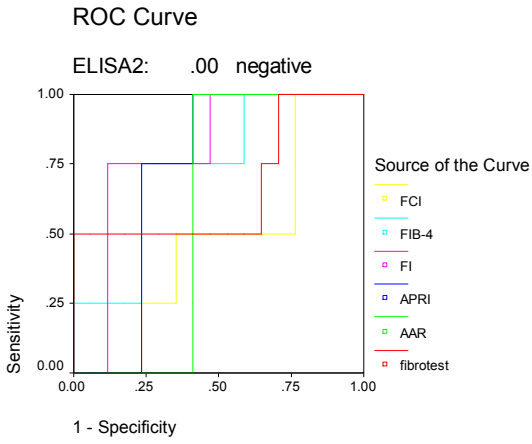
Area Under the Curve

Test Result Variable(s)	Area
fibrotest	.755
AAR	.590
APRI	.675
FI	.706
FIB-4	.677
FCI	.549



Area Under the Curve [a]

Test Result Variable(s)	Area
fibrotest	.769
AAR	.574
APRI	.663
FI	.682
FIB-4	.674
FCI	.576



Area Under the Curve[a]

Test Result Variable(s)	Area
fibrotest	.662
AAR	.588
APRI	.721
FI	.794
FIB-4	.735
FCI	.471

4. Discussion

Aiming to find accurate and reliable non-invasive markers for evaluating fibrosis progression in HCV with or without Co schistosomiasis infection is to avoid the use of invasive liver biopsy. The commonly used markers are: liver function tests (AST, ALT, bilirubin, alkaline phosphatase, albumin and PT). These tests only provide information about important aspects of liver function but they do not assess severity of liver fibrosis or cirrhosis (25). Other serum markers such as α-2-Macroglobulin (26), apolipoprotein A1 (27), haptoglobin (28), are proposed as surrogate indices instead of liver biopsy (29). New researches indicated that these individuals' serum markers have limited accuracy in predicting hepatic fibrosis and proposed that the individual markers are useful for establishing the presence, but not absence, of fibrosis. Due to this limitations, algorithms or indices combining the results of panels of markers have been studied which improve

diagnostic accuracy and proposed as alternatives to liver biopsy (30).

All patients in our study were of genotype 4 to eliminate the HCV genotype effect on fibrosis progression. Co-infection with schistosomiasis in our studied HCV patients was high (79/100) among age group above 40 years which is in agreement with the hypothesis that increased prevalence and intensity of infection with *S. mansoni* in the populous Nile delta where the exposure to canal water was occurring in several million farmers treated with tarter emetic campaigns during 1980s and constituted the major silent reservoirs of HCV (31).

In this study, the impact of schistosomiasis on fibrosis staging was observed from the significant difference of the higher OD absorbance of anti SEA of *S. mansoni* in sever fibrotic and cirrhotic (F3-F4) HCV patients than others (F1-F2) (*P*; 0.001), the linear correlation with fibrosis stages (r; 0.342, *P*; 0.001), and the significant difference of serum α2 macroglobulin levels between F1 and F4.

These findings are in agreement with Silveira *et al.,* (32) who reported increased levels of OD of IgG against SEA in patients with periportal fibrosis. Moreover, studies by Kamal *et al.,* (33) reported that Egyptian patients with co-infections had higher HCV-RNA titers, more advanced liver disease, more hepatic complications, and a greater mortality rate than those with HCV mono infection. Previously, the role of α2 macroglobulin has been discussed by Ahmed et al,(34) as its high levels had an effect on granuloma formation around *S. mansoni* eggs in the rat and it is a reliable predictor of fibrosis in HCV patients. This is could be explained by its association with several growth factors as fibroblast, vascular endothelial, epidermal, transforming and platelet derived growth factors and fibrogenesis (35).

A debate has been raised regarding this role by Shiha and Zalata (36) who concluded that Schistosomal hepatic affection does not alter or interfere with assessment of fibrosis in mixed HCV-Schistosomal liver affection.

We evaluated the performance of AAR, APRI, FIB-4, FCI, FT and FI for staging liver fibrosis and to differentiate them from cirrhosis.

Similar to the poor performance of AAR reported by Lackner *et al.,* (37) our study revealed that it is less accurate in detection of mild fibrosis (F1) among HCV monoinfection and in HCV/*S. mansoni* co-infections. This is in contrary to that reported by Giannini *et al.,* (38), as a high diagnostic accuracy of AAR > 1.16 with 81.3% sensitivity and 55.3% specificity for the prediction of cirrhosis

We observed comparatively low values of APRI (0.58 ± 0.4) in mild fibrosis (F1) of total HCV patients with significant gradual increase in fibrosis stages (*P*; 0.001). APRI was not accurate to detect mild fibrosis (F1) among HCV mono-infection. Khan *et al.,* (39) reported that APRI < 0.42 predict mild fibrosis and > 1.2, predict significant fibrosis in HCV patients with 90% NPV for absence of fibrosis and 91% PPV for fibrosis presence. Our results showed that APRI > 0.46 accurately diagnose fibrosis in HCV/*S, mansoni* co infection patients with 71% sensitivity, 55% specificity. Similarly, Ahmad *et al.,* (34) study revealed low cutoff values with significant direct correlation between APRI and fibrosis stage of the studied Egyptian HCV/*S. mansoni* co infection patients (sig, F2-F4; cutoff 0.60 sensitivity 82%, specificity 57% and F3-F4; cutoff 0.72 sensitivity 94%, specificity 67%).

Our results revealed a cutoff value of < 0.48 FIB-4 in diagnosis of mild fibrosis with sensitivity 62%, specificity 66%, while a cutoff value > 0.5 in the diagnosis of advanced fibrosis has sensitivity of 70%, sp 64%. Although, FIB-4 was not accurate to detect mild fibrosis (F1) among HCV mono-infection, it shows significant correlations of fibrosis and cirrhosis stages in HCV/ *S. mansoni* co infected patients. Similarly, Shaker and Khalifa (40) reported that FIB-4 was reliable in detecting significant fibrosis in Egyptian patients.

Fibrosis index (FI) showed high sensitivity, specificity, and AUROC for discriminating different fibrosis stages among all our studied groups. It was not sensitive to detect mild fibrosis (F1) among HCV mono-infected liver fibrosis stages. Ohta *et al.,* (15) developed this simple index and reported that at cutoff value < 2.1 for predicting F1 stage with 68% sensitivity and 63% specificity. At same cutoff, our data showed comparable results with AUROC 0.614 for the prediction of minimal fibrosis (F1). While for detection cirrhosis in HCV patients, he reported FI value > 3.30, we observed a lower value (> 2.3) with 83% sensitivity and 66% specificity.

Recently, FCI was designed by Ahmad *et al.,* (18) and observed that it could better differentiate among fibrosis stages with high sensitivity, specificity, PPV and NPV. In our study, FCI was not able to detect minimal fibrosis (F1) among HCV mono-infected patients and HCV/ *S. mansoni* co infected patients. However, our lower cutoff values of FCI could be attributed to the inclusion criteria of studied patients who are candidate of interferon/rebaverin treatment as regard platelets ≥90,000 cmm^3 and compensated cirrhosis with normal albumin levels ≥ 3.5 gm/dL.

An interesting finding from the present study was FT showed possibility of classifying all the stages of liver fibrosis with high sensitivity, specificity and AUROC for discriminating different stages among both HCV mono-infected and HCV/ *S. mansoni* co infected patients. El-Shabrawi *et al.,* (41) concluded that a highly significant linear correlation was found between FT-related fibrosis and fibrosis stage by

METAVIR scoring on histopathological examination. On the contrary of this, available data suggest that FT performs well in subjects with grade F1 or F4 of fibrosis, while it performs less well in the intermediate stage (F2). Other studies confirmed the causes of failure of the FT. The most frequent cause leading to false negative result was high haptoglobin in acute inflammation or sepsis. The most frequent cause of false positive results was extremely low haptoglobin associated with intravascular hemolysis and high bilirubin in hemolysis and Gilbert disease (42).

The readily available indexes are associated with some limitations like population discrepancy, not able to distinguish all fibrosis stages individually or some primarily developed for co-infected patients.

Although several non-invasive markers of liver fibrosis have been developed in the last few years, their use in the clinical practice is still limited. In fact, inter laboratory variability, lack of reproducibility and the risk of misdiagnosis (up to 20%), do not allow to recommend these methods in substitution of liver biopsy (43). One of the main limitations for the use of non-invasive markers is the difficult diagnosis of intermediate stages of liver fibrosis (44).

To the best of our knowledge, this is the first time noninvasive biomarkers have been used to assess the feasibility of using six non invasive biomarkers in HCV/*S. mansoni* co infected patients. In this study, we used a single cutoff which is an advantage over other biomarker studies using 2 different cutoffs. Among HCV/*S. mansoni* co-infected patients, FT, FIB-4, FI and FCI can detect all fibrotic and cirrhotic stages (F1, F2-F3,F4) while AAR, APRI can detect fibrotic stages only not the cirrhotic stage. Among HCV mono-infected patients, FT only can detect all fibrotic and cirrhotic stages, FIB-4, FI, AAR and APRI can detect significant fibrosis (F2-F4) and FCI can detect only mild fibrosis (F1). These results suggest that the using FT as a first-line test in the social health centers seems feasible and effective.

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References:

1. Ghany MG, Strader DB, Thomas DL, Seeff LB,2009. Diagnosis, Management, and Treatment of Hepatitis C: An Update. *Hepatology*; 49: 1335–1374.
2. Seeff LB., 2002. Natural history of chronic hepatitis C. *Hepatology*; 36: S35–46.
3. Nafeh M, Abdel Hamid M, Strickland G, *et al.,* 2002. Hepatitis C in a community in Upper Egypt: risk factors for infection. *Am J Trop Med Hyg*,;66(5):633-638.
4. Ray SC, Arthur RR, Carella A, Bukh J, Thomas DL.,2000. Genetic epidemiology of HCV throughout Egypt. *J Infect Dis*, 182:698-707
5. Rao MR, Naficy AB, Darwish MA, Darwish NM, Schisterman E, Clemens JD *et al.,* 2002. Further evidence for association of hepatitis C infection with parenteral schistosomiasis treatment in Egypt. *BMC Infect Dis.*; 2:29-35.
6. Kamal SM, Graham CS, He Q, Bianchi L, Tawil AA, Rasenack JW, *et al.* ,2004. Kinetics of intrahepatic hepatitis C virus (HCV)-specific CD4+ T cell responses in HCV and Schistosoma mansoni coinfection: relation to progression of liver fibrosis. *J Infect Dis.*;189:1140-1150
7. Ghaffar YA, Fattah SA, Kamel M, Badr RM, Mahomed FF, Strickland GT. ,1991. The impact of endemic schistosomiasis on acute viral hepatitis. *Am J Trop Med Hyg*.;45:743–750
8. Berhe N, Myrvang B, Gundersen SG., 2007. Intensity of Schistosoma mansoni, hepatitis B, age, and sex predict levels of hepatic periportal thickening/fibrosis (PPT/F): a large-scale community-based study in Ethiopia. *Am J Trop Med Hyg.*;77:1079-1086.
9. Dienstag JL.,2002. The role of liver biopsy in chronic hepatitis C. *Hepatology*.; 36:S152-160.
10. Bedossa P, Carrat F., 2009. Liver biopsy: The best, not the gold standard. *Journal of Hepatology*.; 50: 1–3.
11. Manning DS, Afdhal NH.,2008. Diagnosis and quantitation of fibrosis. *Gastroenterology*.; 134:1670-1681.
12. Hoofnagle JH, Williams AL., 1988. Ratio of serum aspartate to alanine aminotransferase in chronic hepatitis: relationship to cirrhosis. *Gastroenterology.*; 95:734-739.
13. Wai CT, Greenson JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjeevaram HS *et al.* ,2003. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology*.; 38:518-526.
14. Ngo Y, Munteanu M, Messous D, Charlotte F, Imbert-Bismut F, Thabut D, Lebray P, Thibault V, Benhamou Y, Moussalli J, Ratziu V, Poynard T.,2006. A prospective analysis of the prognostic value of biomarkers (FibroTest) in patients with chronic hepatitis C. *Clin Chem.*, 52:1887-1896.
15. Ohta T, Sakaguchi K, Fujiwara A, Fujioka S, Iwasaki Y, makino Y, *et al.* ,2006. Simple surrogate index of the fibrosis stage in chronic hepatitis C patients using platelet count and serum albumin level. *Acta Med Okayama.*; 60:77-84.
16. Vallet-Pichard A, Mallet V, Nalpas B, Verkarre V, Nalpas A, Dhalluin-Venier V, *et al.* ,2007. FIB-4: an inexpensive and accurate marker of fibrosis in HCV infection. Comparison with liver biopsy and FibroTest. *Hepatology.*; 46:32-36.
17. Silva RG Jr, Fakhouri R, Nascimento TV, Santos IM, Barbosa LM.,2008. Aspartate aminotransferase-to-Platelet ration index for fibrosis and cirrhosis prediction in chronic hepatitis C patients. *Braz J Infect Dis.*; 12:15-19
18. Ahmad W, Ijaz B, Javed FT, Gull S, Kausar H, Sarwar MT *et al.,* 2011. A comparison of four fibrosis indexes in chronic HCV: development of new fibrosis-cirrhosis index (FCI). *BMC Gastroenterol.*; 11:44-53.

19. Ohno O, Mizokami M, Wu RR, Saleh MG, Ohba K, Orito E, *et al.,* 1997. New hepatitis C virus (HCV) genotyping system that allows for identification of HCV genotypes 1a, 1b, 2a, 2b, 3a, 3b, 4, 5a, and 6a. *J Clin Microbiol.*; 35:201-207.
20. Jacobs WH, Goldberg SB.,1989. Statement on outpatient per cutaneous liver biopsy. *Dig Dis Sci*, 34(3):322-323.
21. The French METAVIR Cooperative Study Group,1994. Intra observer and inter observer variations in liver biopsy interpretation in patients with chronic hepatitis C. *Hepatology.*; 20:15-20.
22. Deelder AM., 1973. Immunology of experimental infections with *Schistosoma mansoni* in the Swiss mouse and with *Fasciola hepatica* in the rabbit. *Acta Leiden.*; 39: 105-107.
23. Engvall E, Perlmann P. ,1981. Enzyme-linked immunosorbent assay (ELISA) Quantitative assay of immunoglobulins. *Immunochemistry.*; 8: 871-4.
24. Maor Y, Calès P, Bashari D, Kenet G, Lubetsky A, Luboshitz J, *et al.* ,2007. Improving estimation of liver fibrosis using combination and newer noninvasive biomarker scoring systems in hepatitis C-infected haemophilia patients. *Haemophilia.*; 13:722-729.
25. Rossi E, Adams LA, Bulsara M, Jeffrey GP., 2007. Assessing Liver Fibrosis with Serum Marker Models. *Clin Biochem Rev.*; 28: 3–10.
26. Naveau S, Poynard T, Benattar C, Bedossa P, Chaput JC., 1994. Alpha-2-macroglobulin and hepatic fibrosis. Diagnostic interest. *Dig Dis Sci.*; 39: 2426–2432.
27. Poynard T, Aubert A, Bedossa P, Abella A, Naveau S, Paraf F, *et al.,* 1991. A simple biological index for detection of alcoholic liver disease in drinkers. *Gastroenterol.*; 100: 1397–1402.
28. Bismut F, Ratziu V, Pieroni L, Charlotte F, Benhamou Y, Poynard T. Multivirc Group,2001. Biochemical markers of liver fibrosis in patients with hepatitis C virus infection: a prospective study. *Lancet.*; 357:1069-1075.
29. Gressner OA, Weiskirchen R, Gressner AM., 2007. Biomarkers of liver fibrosis: Clinical translation of molecular pathogenesis or based on liver-dependent malfunction tests. *Clinica Chimica Acta.*; 381: 107-113.
30. Crockett SD, Kaltenbach T, Keeffe EB., 2006. Do we still need a liver biopsy? Are the serum fibrosis tests ready for prime time? *Clin Liver Dis.*; 10: 513-534.
31. Strickland GT. ,2006. Liver disease in Egypt: hepatitis C superseded schistosomiasis as a result of iatrogenic and biological factors. *Hepatology.*;43:915-922
32. Silveira AM, Bethony J, Gazzinelli A, Kloos H, Fraga LA, Alvares MC *et al.,* 2002. High levels of IgG4 to *Schistosoma mansoni* egg antigens in individuals with periportal fibrosis. *Am J Trop Med Hyg.*; 66:542-549.
33. Kamal S, Madwar M, Bianchi L, Tawil AE, Fawzy R, Peters T, *et al.* ,2000. Clinical, virological and histopathological features: long-term follow-up in patients with chronic hepatitis C co-infected with *S. mansoni*. *Liver.*; 20:281-289.
34. Ahmed L, Salama H, Ahmed R, Hamdy S, Al-Akel W, Shafi S A *et al.,* 2009. Non-invasive fibrosis seromarkers as a predictor of liver fibrosis in chronic hepatitis C and/or non-alcoholic steatohepatitis. *Arab Journal of Gastroenterology.*; **10**: 14–20.
35. Sakai K, Lowman HB, Clemmons DR., 2002. Increases in free, unbound insulin-like growth factor I enhance insulin responsiveness in human hepatoma G2 cells in culture. J Biol Chem.; 277:13620-13627.
36. Shiha G, Zalata KR., 2002. Does schistosomiasis interfere with application of the Knodell score for assessment of chronic hepatitis C*? Med Sci Monit.*; 8: CR 72-77.
37. Lackner C, Struber G, Liegl B, Leibl S, Ofner P, Bankuti C *et al.,* 2005. Comparison and validation of simple noninvasive tests for prediction of fibrosis in chronic hepatitis C. *Hepatology.*; 41:1376-1382.
38. Giannini E, Risso D, Botta F, Chiarbonello B, Fasoli A, Malfatti F *et al.,* 2003. Validity and clinical utility of the aspartate aminotransferase-alanine aminotransferase ration in assessing disease severity and prognosis in patients with hepatitis C virus related chronic liver disease. *Arch Intern Med.*; 163:218-224.
39. Khan DA, Fatima-Tuz-Zuhra , Khan FA, Mubarak A., 2008. Evaluation of diagnostic accuracy of apri for prediction of fibrosis in hepatitis c patients. *J Ayub Med Coll Abbottabad*,; 20:122-126.
40. Shaker MK, Khalifa MO., 2012. Comparison between different noninvasive fibrosis seromarkers and liver biopsy in staging fibrosis in Egyptian patients with chronic hepatitis C virus Infection. *Egyptian Liver Journal.* ; 2: 1215.
41. El-Shabrawi MH, Mohsen NA, Sherif MM, El-Karaksy HM, Abou-Yosef H, El-Sayed HM *et al.,* 2010. Noninvasive assessment of hepatic fibrosis and necroinflammatory activity in Egyptian children with chronic hepatitis C virus infection using FibroTest and ActiTest. *Eur J Gastroenterol Hepatol.*; 22:946-951.
42. Bongiovanni M, Casana M. ,2008. Non-invasive markers of liver fibrosis in HCV mono-infected and in HIV/HCV co-infected subjects. *Med Chem.*; 4:513-519.
43. Munteanu M, Ratziu V, Morra R, Messous D, Imbert-Bismut F, Poynard T. ,2008. Noninvasive biomarkers for the screening of fibrosis, steatosis and steatohepatitis in patients with metabolic risk factors: FibroTest-FibroMax experience. *J Gastrointestin Liver Dis.*; 17:187-191.
44. Gebo KA, Herlong H.F.; Torbenson M.S.; Jenckes M.W.; Chander G.; Ghanem K.G.; *et al.,* 2002. Role of liver biopsy in management of chronic hepatitis C: a systematic review. *Hepatology*.; 36: S161-172.

2/2/2012