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# SERUM MARKERS FOR ASSESSING LIVER FIBROSIS IN EGYPTIAN PATIENTS WITH CHRONIC HEPATITIS B AND C CO-INFECTION VERSUS CHRONIC HEPATITIS C

By

**LAMIAA MOBARAK**

National Hepatology and Tropical Medicine Research Institute, Cairo, Egypt

(lamiaamobarak@yahoo.com, lamiaamobarak@hotmail.com)

## Abstract

Chronic hepatitis B and C can progress to hepatic fibrosis and cirrhosis. The stage of liver fibrosis is critical for decision of treatment and prediction of outcomes, as life threatening complications highly develop in cirrhotic patients. The aim of this study was to evaluate the diagnostic accuracy of non-invasive serum markers in the assessment of liver fibrosis in patients with combined chronic hepatitis B and C versus those with chronic hepatitis C.

This study included 2 groups; G1: combined chronic hepatitis B & C, which included 71 patients and G2: chronic hepatitis C, which included 70 patients. Liver biopsy results from both groups were recorded. Three validated blood indices Fibro Q, Fibro alpha, and Biotechnology Research Center (BRC) were tested for optimal cut off values for assessing liver fibrosis in both groups.

The results showed that the area under receiver operating characteristic curves (AUROC) for Fibro Q in differentiating significant fibrosis (>F2) from non-significant fibrosis (≤F2) was 0.79 (95% CI: 0.60-0.89) in the first group and 0.85(95% CI: 0.71-0.98) in the second group. AUROC for BRC was 0.76 (95% CI: 0.63-0.89) in the first group and 0.75 (CI: 0.60-0.89) in the second group. Fibro alpha performed less in both groups based on AUROC 0.69 and 0.68 in the first and second group respectively.

**Key words**: Combined HBV/HCV, Chronic HCV, Liver fibrosis-non-invasive, Serum markers.

## Introduction

Chronic hepatitis B virus (HBV) and C virus (HCV) represent significant issues of public health worldwide (Konstanitinon and Deutsch, 2015). Prevalence of HBV/HCV co-infection is unknown and might be underestimated with silent phenomenon (occult) HBV infection (Chi-Jen and Shou-Dong, 2008). Co-infection of HBV & HCV is common in highly endemic areas as Egypt (Konstanitinon; Deutsch, 2015). These patients have an increased risk of progression to cirrhosis and decompensated liver disease (Lee *et al*, 2007; Sagnelli *et al*, 2009), and a higher risk of hepatocellular carcinoma (HCC) (Donato *et al*, 1998; Shi *et al*, 2005).

Egypt has the highest prevalence rates of virus C; an estimated 10-15% of the population, about 8-10 million people, carried hepatitis C antibodies. About five million of them were actively infected (Hussein, 2014).

Liver fibrosis assessment is crucial for early management of patients with chronic hepatitis B (CHB) and/or chronic hepatitis C (CHC). However, the lack of an easy, accurate method to be applied in clinical practice remains the major obstacle. In spite of the fact that liver biopsy is the gold standard for liver fibrosis staging; it is invasive and expensive with risk of sampling error and variability in pathological interpretation (Baranova *et al,* 2011).

Transient elastography has been approved and shown to be superior to FIB-4 in diagnosis of advanced fibrosis (F3–F4) and cirrhosis (Castera, 2012; Verv-eer *et al*, 2012). However, in comparison with serum markers, it was expensive, less helpful in diagnosing early fibrosis and less easily available (Holmberg *et al*, 2013). Several serum markers were validated for liver fibrosis assessment, but without greatly applied in CHB (Adler *et al*, 2008; Kim *et al,* 2010).

In the present study, three readily available serum fibrosis markers were used to test the optimal cut off values to distinguish between early fibrosis and advanced fibrosis among two groups of patients; G1 with combined

chronic hepatitis B and C and G2 with only chronic hepatitis C, to evaluate the diagnostic accuracy of non-invasive serum markers in the assessment of liver fibrosis in both groups of patients.

## Patients, Materials and Methods

This prospective study included two groups; G1: 71 Egyptian patients with combined chronic hepatitis B & C and G2: 70 patients with chronic hepatitis C, presented to out-patient clinics of National Hepatology and Tropical Medicine Research Institute.

Diagnosis of chronic liver disease was based on clinical, laboratory and imaging evidence of chronic liver disease. Diagnosis of HCV was based on detection of HCV antibodies. Diagnosis of HBV was based on detection of HBs Ag. All patients with decompensated liver disease, hepatocellular carcinoma, other causes of chronic liver disease, history of previous antiviral therapy, Body mass index (BMI) >30 and presence of absolute contraindication for liver biopsy were excluded from this study.

An informed written consent was obtained from all patients and the study was according to the Declaration of Helsinki.

All patients were subjected to detailed history, thorough clinical examination, and basic laboratory tests including: Complete blood picture (CBC), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), serum albumin, total bilirubin, INR, Alpha fetoprotein (AFP), hepatitis seromarkers for HCV (anti HCV) and for hepatitis B virus (HBV); (HBsAg, anti-HBc & anti-HBs) using ELISA technique. HCV RNA was tested to all patients, while HBV DNA was tested to all patients in G1, by quantitative PCR. Then all patients were subjected to abdominal ultrasound and evaluated for Fibro Q, Fibro alpha, and Biotechnology Research Center (BRC) indices.

The following formulas were used: Fibro Q = [(10×age (years)×AST×PT- INR)/ (PLT ×ALT)] (Hsieh *et al,* 2009), Fibro alpha =1.3+AFP (U/L) x 0.009584 +AARx 0.243 - platelet count x 0.001624 (Omran *et al,* 2011) and Biotechnology Research Center (BRC) score=1.02+0.4xAFP (U/L)+0.19x Age (years)- 0.02 x platelet count ($10^9$/l) (Attalah *et al,* 2013).

Ultrasound guided liver biopsies were performed within a week of liver fibrosis assessment. All samples were examined by a single pathologist, blind to the results of the serum markers. Fibrosis was staged according to the METAVIR scoring system from F0 to F4 as: F0 (no fibrosis), F1 (mild fibrosis without septa), F2=moderate fibrosis with few septa, F3=severe fibrosis with numerous septa without cirrhosis and F4= cirrhosis (Bedossa and Poynard, 1996). Histopathologically, liver biopsies of patients, with combined chronic hepatitis B & C were divided into two groups: significant fibrosis group (F>2; 56 patients), and without significant fibrosis group (F≤2; 15 patients). Patients with chronic HCV showed significant fibrosis (F> 2; 37 patients), and without significant fibrosis (F≤ 2; 33 patients).

Statistical analysis: Data were presented as means ± standard deviation (S.D), while categorical data were presented as number (percent). The Mann-Whitney U test and the Chi-square test were used when appropriate. Statistical significance was considered if P value was less than or equal 0.05. Diagnostic performance of non-invasive serum markers were assessed by measuring the area under the receiver-operating characteristics (AUROC). Diagnostic accuracy was evaluated by comparing sensitivity, specificity, positive and negative predictive values (PPV and NPV respectively).

## Results

The HBV/HCV co-infected patients in relation to fibrosis stage, mean age with significant fibrosis (>F2) was 49±11.6, patients with early fibrosis (≤F2) was 38±10.3. Male predominance was (71.4% & 80%) in cases with significant fibrosis and early fibrosis respectively. Tansaminases were mildly elevated (<3 folds). Serum albumin, INR, and platelets count were significantly worse between both groups respectively. Fibrosis

scores Fibro Q, Fibro alpha, & BRC were significantly higher between grou-ps (P= 0,001, 0.02 & 0.02) respectively.

Fibro Q and BRC showed higher diagnostic performance in prediction of significant fibrosis based on (AUROC of 0.79, CI; 0.60-0.89 & 0.76, CI; 0.63-0.89) respectively compared to fibro alpha with AUROC (0.69; 0.56-0.82). Fibro Q, BRC, and fibro alpha showed cut off values of 2.07, 6.3, and 1.3 for prediction of significant fibrosis.

HCV group in relation to fibrosis stage, mean age of patients with significant fibrosis (>F2) was 39±9.5, mean age with early fibrosis (≤F2) was 46±12.7 with significant difference (P=0.02). There was male predominance (59.4% & 60.6%) in significant fibrosis and early fibrosis respectively. Serum albumin, INR, & total bilirubin gave significant difference between groups.

Fibrosis scores: Fibro Q & BRC were significantly higher between groups (P= 0,001, and 0.01) respectively. Fibro Q & BRC showed good diagnostic performance in prediction of significant fibrosis based on (AUROC of 0.85, CI; 0.71-0.98 and 0.75, CI; 0.60-0.89) respectively compared to fibro alpha with AUROC (0.68; 0.50-0.86). Fibro Q, BRC, & fibro alpha showed cut off values of 4.5, 8.1, and 1.4 for prediction of significant fibrosis. Details were given in tables (1, 2, 3 & 4) and figures (1 & 2).

Table 1: Combined HCV and HBV group in relation to fibrosis stage

| | >F2 (n=56) | ≤ F2 (n=15) | P value |
|---|---|---|---|
| Age | 49±11.6 | 38.4± 10.3 | 0.001* |
| Males | 40 (71.4%) | 12 (80%) | 0.5 |
| Females | 16 (28.6) | 3 (20%) | |
| HCV PCR | $2.5x10^5$ (less than 16-$1.6x10^6$) | 1 x $10^5$ (less than 16-$4x10^7$) | 0.6 |
| HBV PCR | 2784 (less than 84 – 2 $x10^7$) | 3085 (less than 84 – 4 $x10^8$) | 0.3 |
| Total Bilirubin | 0.7± 0.3 | 0.8± 0.6 | 0.7 |
| AST | 50 ( 12-118) | 35 (7-147) | 0.1 |
| ALT | 74 (11-191) | 40 (6-191) | 0.07 |
| ALB | 3.8± 0.5 | 4±0.5 | 0.005* |
| INR | 1.2± 0.2 | 1.09± 0.1 | 0.005* |
| WBC | 6.5± 3 | 6.0± 2.0 | 0.5 |
| HB | 13.7± 3.1 | 13.5± 1.6 | 0.4 |
| PLT | 140± 59 | 198± 57 | 0.001* |
| AFP | 3.6 (0.6-319) | 2.5 (0.7- 65.4) | 0.3 |
| Fibro Q | 3.8 (1.1-12.3) | 1.9 (0.5-6.7) | 0.001* |
| Fibro Alpha | 1.4 (1.2-4.4) | 1.2 (1-2.2) | 0.02* |
| BRC | 9.1 (2.4-139) | 5 (0.8-31.3) | 0.02* |

* P value≤ 0.05 =significant

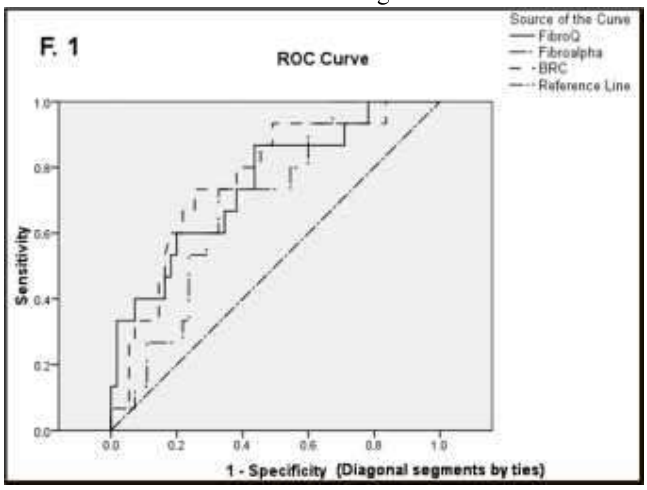


Fig. 1: ROC curve for Fibro Q, Fibro alpha and BRC in combined HCV and HBV group

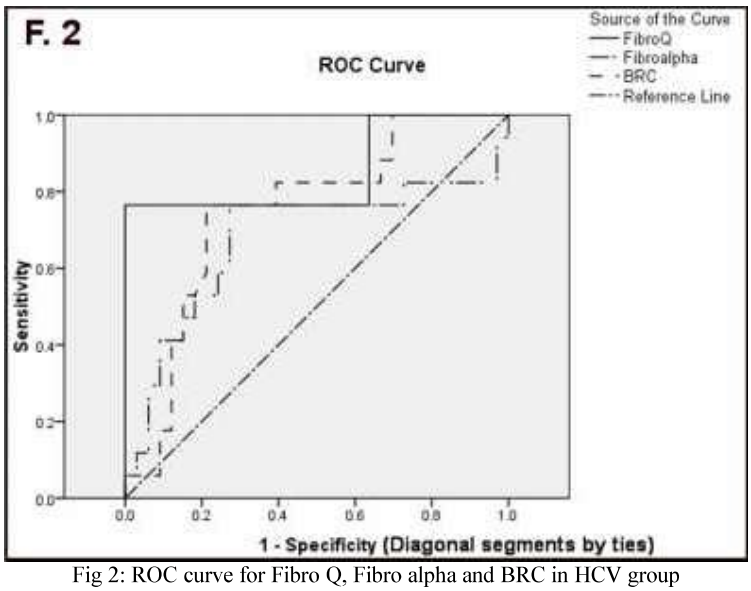

Fig 2: ROC curve for Fibro Q, Fibro alpha and BRC in HCV group

Table 2: Accuracy measures of Fibro Q, Fibro alpha & BRC to diagnose advanced fibrosis in HCV & HBV group.

| | Cut off | SEN | SPE | PPV | NPV | LR (+) | LR(-) | AUC | 95% CI |
|---|---|---|---|---|---|---|---|---|---|
| Fibro Q | 2.07 | 86.7% | 56.4% | 66.3% | 80% | 1.9 | 0.2 | 0.75 | 0.60-0.89 |
| Fibro alpha | 1.3 | 73.3% | 67.3% | 69.3% | 71.6% | 2.2 | 0.4 | 0.69 | 0.56- 0.82 |
| BRC | 6.3 | 80.% | 61.8% | 67.8% | 75.8% | 2.1 | 0.3 | 0.76 | 0.63-0.89 |

LR(+), positive likelihood ratio; LR(−), negative likelihood ratio; NPV, negative predictive value; PPV, positive predictive value; SEN, sensitivity; SPE, specificity, AUC (area under curve). Biotechnology Research Center (BRC).

Table 3: HCV group in relation to Fibrosis stage

| | ≤ F2 (n= 33) | >F2 (n= 37) | P value |
|---|---|---|---|
| Age | 46.8±12.7 | 39± 9.5 | 0.02* |
| Male | 20 (60.6%) | 22 (59.4%) | 0.9 |
| Female | 13 (39.4%) | 15 (40.6%) | |
| Total Bilirubin | 0.6± 0.2 | 0.7± 0.3 | 0.04 |
| AST | 50.4 ± 23.4 | 51.7 ± 18.4 | 0.8 |
| ALT | 51± 26 | 59 ± 22 | 0.2 |
| ALB | 4± 0.3 | 4.2±0.2 | 0.01* |
| INR | 1.1± 0.1 | 1.04± 0.07 | 0.03* |
| WBC | 6.9± 2.4 | 7± 1.8 | 0.8 |
| HB | 13.5± 1.4 | 14.3± 1.3 | 0.4 |
| PLT | 216± 61 | 198± 70 | 0.3 |
| HCV PCR | $4.3x10^5$ (64 - $2x10^7$) | $6.2 x 10^5$ ($9354-4x10^6$) | 0.6 |
| AFP | 4.2 (1.2-27) | 4.4 (1.3- 27) | 0.3 |
| Fibro Q | 2.7 (0.5-3.9) | 5.3 (1.7-12.4) | 0.001* |
| Fibro Alpha | 1.3 (1.1-1.6) | 1.4 (1.1-1.6) | 0.09 |
| BRC | 7.1 (1.6-17.6) | 10.4 (4.7-21.3) | 0.01* |

* P value≤ 0.05 significant

Table 4: Accuracy measures of Fibro Q, Fibro alpha & BRC to diagnose advanced fibrosis in HCV group.

| Items | Cut off | SEN | SPE | PPV | NPV | LR (+) | LR (-) | AUC | 95% CI |
|---|---|---|---|---|---|---|---|---|---|
| Fibro Q | 4.5 | 76.5% | 100% | 100% | 80.5% | | 0.2 | 0.85 | 0.71-0.98 |
| Fibro alpha | 1.4 | 76.5% | 72.7% | 73.6% | 75% | 2.7 | 0.3 | 0.68 | 0.50- 0.86 |
| BRC | 8.1 | 82.4% | 60.6% | 67.4% | 77.7% | 2.0 | 0.2 | 0.75 | 0.60-0.89 |

## Discussion

Generally, HBV/HCV co-infected persons suffered more severe liver injury, a higher probability of liver cirrhosis and hepatic de-compensation, and a higher incidence of hepatocellular carcinoma (Chi-Jen, and Shou-Dong, 2008).

Although liver biopsy is yet considered the most accurate method for fibrosis staging in chronic hepatitis B or C, it is invasive, with potential serious effects and limitations (Seeff *et al*, 2010; West and Card, 2010). This pushes the demand for noninvasive tests as alternative to biopsy. Many blood tests for fibrosis and cirrhosis were found with AUROC range 0.70 to 0.86 and range 0.80 to 0.91 respectively (Zweig and Campbell, 1993; Altman and Bland, 1994).

The present study presented an additional feature, fibrosis assessment using three serum markers in patients with combined chronic hepatitis B & C versus patients with chronic hepatitis C only.

In the present study, significant fibrosis (>F2) was presented in 56/71 of patients with combined chronic HBV/HCV compared to only 37/70 of patients with chronic HCV. This agreed with the fact that co-infection with HBV/HCV being at higher risk for progression to cirrhosis (Chou and Wasson, 2013). There was male predominance in both groups of patients with no statistically significant difference. This agreed with the fact that female gender is favored for HCV clearance (Bakr *et al,* 2006).

In the present study chronic HBV/ HCV co-infected patients, labs reflected hepatocyte synthetic function i.e., albumin, bilirubin and INR were significantly worse in significant fibrosis patients than those without significant fibrosis. Hepatic enzymes reflecting inflammatory process, particularly AST, ALT, & AFP were higher in patients with significant fibrosis than those without significant fibrosis, however did not show significant difference. While in chronic HCV patients, total bilirubin was higher in significant fibrosis patients than those without significant fibrosis. Also AST, ALT, & AFP were slightly higher in patients with significant fibrosis than those without significant fibrosis but without significant difference.

In the present study, platelets count in both groups was lower in patients with significant fibrosis than those without significant fibrosis, accordingly platelet count could be a surrogate marker to predict the stage of fibrosis, particularly at F3 and F4 (Khokhar, 2003).

In this study, cut off value 2.07 & 4.5, Fibro Q significantly diagnoses fibrosis (F>2) with AUROCs of 0.75 and 0.85 for combined CHB & CHC and CHC respectively. Fibro Q was identified by significant fibrosis (Hsieh *et al,* 2009).

At cut off 6.3 and 8.1, BRC can diagnose significant fibrosis (F> 2) with AUROC of 0.76 and 0.75 for combined CHB & CHC and CHC respectively. Attalah *et al*. (2013) reported that BRC score performed better than Fibro-α score as a novel non-invasive test to evaluate liver fibrosis in CHC Egyptian patients. While, at cut off 1.3 & 1.4, fibro alpha can diagnose significant fibrosis (F> 2) with AUROC of 0.69 & 0.68 for combined CHB & CHC and CHC respectively, which were lower than reported in a previous study that showed the diagnostic accuracies of Fibro-α for significant fibrosis, advanced fibrosis, and cirrhosis were 0.74, 0.82 & 0.80, respectively (Omran *et al,* 2011).

In the present study, applying fibro Q, Fibro-α and BRC scores on combined chronic HBV/HCV showed significant diagnostic value for fibrosis (>F2) as compared to fibro alpha for both groups combined chronic HBV/HCV & CHC.

## Conclusion

No doubt, chronic hepatitis C & B and their complications are health problem. The Fibro Q & BRC scores proved to be better than fibro alpha in predicting fibrosis, and assessing liver fibrosis in chronic hepatitis B & C co-infection as well as in chronic hepatitis C, with a dependable diagnostic accuracy in chronic hepatitis C.

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