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***Corresponding author:**
Hussien Mohamed;
Email: aljebouri_999@hotmail.com

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Diagnostic Value of Fibrotic Indices in Egyptian Patients with Chronic Hepatitis and Hepatocellular Carcinoma

Mohamed Hussien[1]*, Faten Zahran[2], Hoda El-Emshaty[3], Mona Samy Gouida[4], Muhammad Naeem Iqbal[5,6]

[1]Mansoura Chest Hospital, Mansoura, Egypt.
[2]Department of Chemistry, Faculty of Science, Zagazig University, Zagazig, Egypt.
[3]Gastroenterology Center, Mansoura University, Mansoura, Egypt.
[4]Mansoura Children Hospital, Mansoura University, Mansoura, Egypt.
[5]The School of Life Sciences, Fujian Agriculture and Forestry University, Fuzhou 350002, China.
[6]Pakistan Science Mission (PSM), Noor Kot 51770, Pakistan.

Abstract
Cirrhosis and hepatocellular carcinoma (HCC) end-stages of chronic liver diseases (CLD) are leading causes of morbidity and mortality worldwide. The main aetiologies of CLDs are chronic hepatitis C virus (HCV) infection. The aim of this work was to evaluate fibrotic indices APRI and FIB-4 in patients with chronic liver disease (CLD) with HCC, Cirrhosis, and healthy individuals. In the present study, we have investigated 30 Hepatocellular Carcinoma (HCC) patients, from Gastroenterology Center, Mansoura University, compared with 17 cirrhotic patients, and 25 normal healthy individuals as a control group. Laboratory liver fibrosis indices including FIB-4, APRI, and AST/ALT ratio were calculated in Egyptian patients with chronic hepatitis C virus. Results from this study were high APRI and FIB-4 in HCC patients compared with Cirrhosis and Control groups with significance difference ($p<0.05$). Based on our observation in this study APRI and FIB-4 which representing fibrotic indices have diagnostic value in the assessment of hepatocellular carcinoma.
Keywords: Chronic Liver Diseases (CLD); HCV; Hepatocellular Carcinoma (HCC); AFP.

INTRODUCTION

Hepatitis C virus (HCV) infection is a global public health burden. Approximately 170 million people are infected with HCV worldwide, and most of these patients become persistently infected. Furthermore, HCV infection in some patients may progress into chronic liver diseases, such as steatosis, cirrhosis, and hepatocellular carcinoma (Choo *et al*., 1989; Ali *et al.,* 2015). HBV and HCV achieved an endemic situation in many countries of the world (Kalim *et al.,* 2017). It is reported that elevation of liver enzymes is responsible for liver disorders (Toor *et al.,* 2016). The chronic liver abnormalities are often accompanied by indigestion, fatigue, weakness, fever, abdominal pain, nausea, and loss of appetite (Muhammad *et al.,* 2013). In Egypt, hepatocellular carcinoma (HCC) is the second most common cancer in men and the 6th most common cancers in women. Hospital-based studies from Egypt have reported an overall increase in the relative frequency of all liver-related cancers in Egypt, from approximately 4% in 1993 to 7.3% in 2003. This rising incidence (Lehman *et al*., 2008) may be due to the high prevalence of hepatitis C virus (HCV) and its complications and the fact that people born 20 years ago or earlier in Egypt has not been vaccinated against hepatitis B virus (HBV) (Yates *et al.,* 1999). Because of the prevalence, HCC is the most studied primary liver cancer. Several different histological subtypes are known such as scirrhous HCC, fibrolamellar carcinoma, combined HCC-Cholangiocarcinoma (HCC-CC), sarcomatoid HCC, undifferentiated carcinoma, lymphoepithelioma-like HCC, clear cell HCC, diffuse cirrhosis-like HCC, steatohepatitis HCC, transitional liver cell tumor, and CAP carcinoma (Roncalli *et al.,* 2010). The lack of useful molecular markers to classify HCC aggressiveness hereby complicates clinical analyses to stage patient's outcomes (Okuda, 2000). Development of liver tumors and their evolution to HCC is a multi-step process where different HCC-etiologies provoke continuous rounds of hepatocytes damage and regeneration. These cycles of damage-death-regeneration lead to collagen accumulation contributing to liver fibrosis. Over an extended time, this triggers a cirrhotic state considered as a pathological state of the liver whose lesions can progress to a pre-malignant state producing dysplastic nodules. Later, these nodules will evolve to HCC invading the surrounding stroma and occasionally generating metastatic events (Borzio *et al*., 1995). Transcriptional analyses of liver tumors revealed alterations of several molecular pathways during cancer development implicated in cell proliferation, cell cycle regulation, apoptosis, (Hanahan and Weinberg, 2011) angiogenesis, cell signaling, metabolism, and immune response (particularly in HCC with HBV/HCV infection). Investigations in Egypt have shown the increasing importance of HCV infection in the etiology of liver cancer, estimated to account for 40-50% of cases, and the declining influence of HBV and HBV/HCV infection (25% and 15%, respectively). The rising incidence of HCC in Egypt could be explained through improvements in screening programs and diagnostic tools, as well as the increased survival rate among patients with cirrhosis allowing time for some of them to develop HCC (El-Serag, 2001). The aim of this study was to determine diagnostic significance of fibrotic indices in Egyptian patients with chronic hepatitis and hepatocellular carcinoma.

MATERIALS AND METHODS

Study design

This study consisted of a total of 30 HCC patients recruited from Gastroenterology Center, Mansoura University were enrolled in this study represented the group I. They were diagnosed as HCC according to clinical examination, radiological investigations including abdominal ultrasonography, triphasic C.T, and laboratory investigations. There were 17 Cirrhotic patients representing group II. Additionally, healthy 25 subjects were enrolled in this study represented group III; they had normal values of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and were seronegative for hepatitis B surface markers and HCV antibodies, HIV.

Ethical approval

The protocol was conducted in accordance with guidelines approved by local research ethics committee and the subjects in this study were matched in regard to sex and age and informed consent was obtained from all the patients and volunteers.

Collection of samples and biochemical analyses

Six milliliters of venous blood specimens were collected in dry clean centrifuge tubes after an overnight fast, left to clot for 30 minutes at 37°C, and then centrifuged at 3000 rpm for 10 minutes. The sera were then separated, divided into several aliquots, stored at -20°C to be thawed only once on demand for the biochemical analyses. CBC was carried out using automated cell counter Sysmex KX-21N (TAO Medical incorporation, Japan). All patients and controls were subjected to full clinical assessment. Laboratory investigations included aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), Alpha-fetoprotein (AFP), total bilirubin, and serum albumin. All were performed on Beckman CX9 autoanalyzer.

Fibrotic Indices Calculation

APRI = (AST / upper limit of normal AST) × 100 / platelet count) (Wai *et al*., 2003).

FIB-4 = (AST × age / platelet counts × ALT $^{1/2}$) (Sterling *et al*., 2006).

Statistical analysis

A computer software package (SPSS), version 16.0 was used in the analysis. For quantitative variables, mean and median (as a measure of central tendency), the standard deviation was used. Frequency and percentage were presented for qualitative variables. Significance level (p) value was expressed as follows: $p > 0.05$ = Insignificant, $p < 0.05$ = Significant and $p < 0.001$ = highly significant.

RESULTS

Our results demonstrated that among 30 HCC patients, 23 (76.6%) were males and 7 females (23.3%), and their age ranged from 45-69 years with a mean of 55.6±6.92 years. Among 17 patients with cirrhosis representing group II, 13 (76.5%) were males and 4 females (23.5%), and their age ranged from 53-87. Among 25 unrelated healthy adults representing group III with no liver diseases as a control group, 17 (68.0%) were males and 8 females (32.0%), and their age ranged from 23-37 years with a mean of 26.8±3.014 years as shown in (Table 1). Serum ALT and AST were higher in Cirrhotic patients comparing with HCC patients and control group with significance difference ($p< 0.05$). Serum APRI and FIB-4 indices were higher in HCC patients compared with Cirrhotic and Control groups with significance difference ($p< 0.05$) as shown in (Table 2). Platelet count was higher in control group than patients (Figure 1). A previous study showed the levels of biochemical markers (AST, ALT, and bilirubin) of the liver fibrosis group were higher than healthy individual's groups ($p < 0.001$) (Canbakan *et al*., 2009). Giannini et al. (2006) found that AST/ALT ratios increased and platelet counts decreased as liver fibrosis worsened. The rates of HCC are consistently higher for males than females, but the male to female ratio differs with the country as well as with the year of survey, because of the changing time trends. In our study, HCC males were (76.6 %) while females with HCC were (23.3 %). The incidence of HCC increases progressively with age, although this varies by country. Thus, in high incidence countries, the mean age at the time of diagnosis is in the third decade of life, and in low incidence countries, it occurs 2 to 3 decades later. In our study, the mean age of HCC patients, Cirrhotic patients, and healthy control was 55.6 ± 6.92, 65.1 ± 11.7 and 26.8 ± 3.01 years, respectively. There was a significant difference between groups regarding the age distribution (Table 1). This finding was close to previous research by Massoud et al. (2006). In the present study, there was a significant positive correlation between ALT and AST ($r=0.789$, $p<0.05$) (Figure 2). APRI and FIB-4 were also found to have a significant positive correlation ($r= 0.906$ and $P<0.05$) (Figure 3).

Table 1. Descriptive data in HCC, Cirrhosis and Control groups

Variables	HCC Patients	Cirrhotic Patients	Healthy Control
Number	N= 30	N= 17	N= 25
Age mean ± S.D	55.6 ± 6.92	65.1 ± 11.74	26.8 ± 3.014
Range	45-69	53-87	23-37
Male	23 (76.6 %)	13(76.5%)	17 (68.0 %)
Female	7 (23.3 %)	4(23.5%)	8 (32.0 %)

Table 2. Mean and S.D of biochemical parameters and fibrotic indices in HCC, Cirrhosis and Control groups

Variables	HCC mean ± S.D	Cirrhotic mean ± S.D	Control mean ± S.D
ALT (40 IU/L)	53.1 ± 41.2	65.9 ± 34.8	14.04 ± 3.39
AST(40 IU/L)	65.3 ± 29.1	72.6 ± 20.0	15.6 ± 2.30
AST/ALT index	1.59 ± 0.96	1.28 ± 0.54	1.14 ± 0.30
APRI index	1.5 ± 1.03	0.79 ± 0.51	0.12 ± 0.03
FIB-4 index	4.8 ± 2.73	2.33 ± 1.15	0.36 ± 0.12

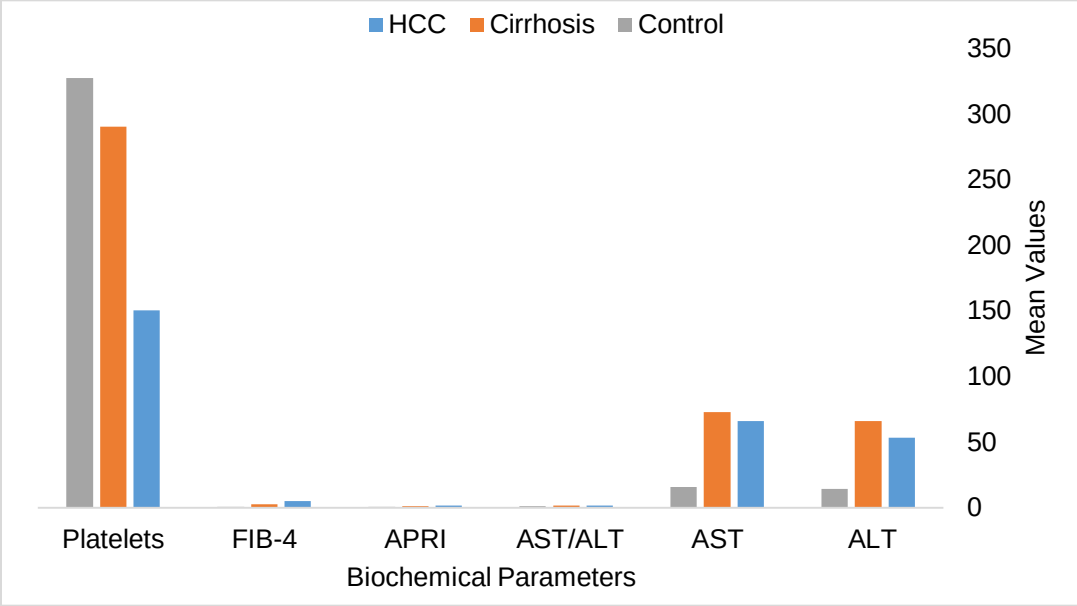

Fig. 1. Mean values of biochemical parameters and fibrotic indices in all groups.

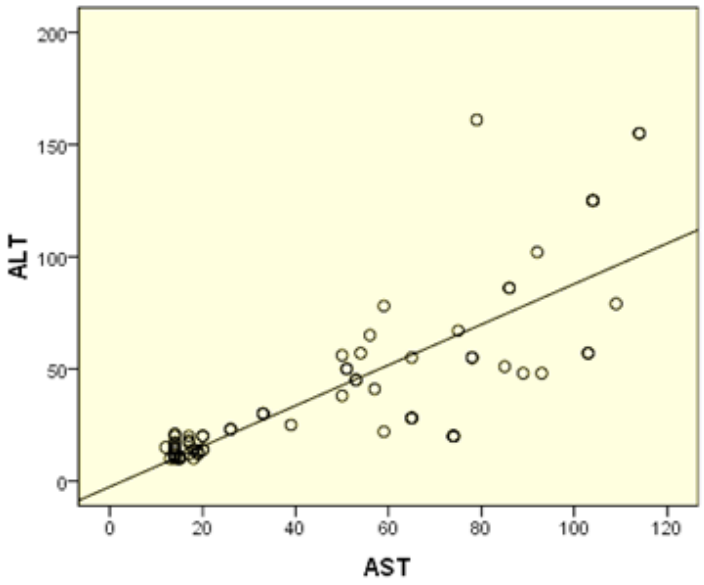

Fig. 2. Pearson Correlation between AST and ALT

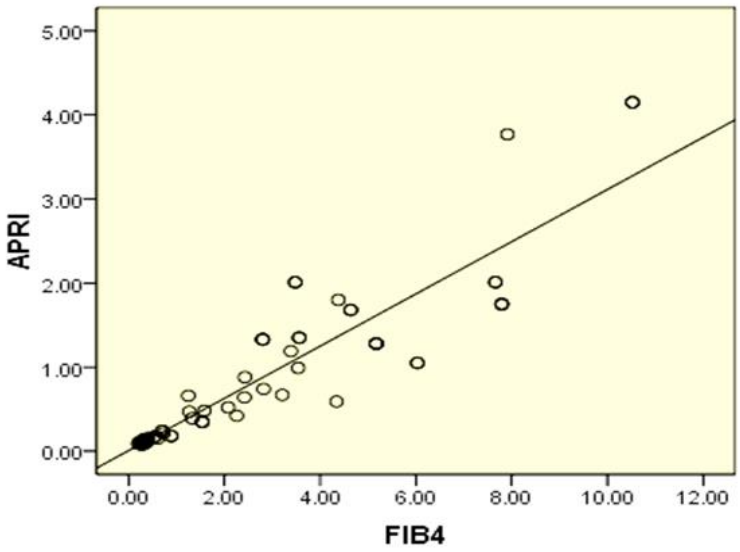

Fig. 3. Pearson Correlation between APRI and FIB-4

In this study there was a significant difference between the concentration of AFP in both HCC patients and Control, there was a high concentration of AFP in HCC patients with mean 4.78 ±7.42 and ranged between 0.8 – 2000 ng/ml compared with healthy control groups with mean 2.7±0.55 and ranged between 1.5-4.0 ng/ml (Table 3). In our study for detection of specificity and sensitivity, ROC was estimated and AUROC was (0.8, p <0.05) (Figure 4). Alpha-fetoprotein (AFP) is a glycoprotein with a size of 591 amino acids. It is normally synthesized during fetal life, first in the yolk sac and then in the fetal liver; its synthesis is normally repressed in adults. High levels of AFP are observed during adulthood only under certain conditions, such as pregnancy, the presence of some neoplasias (e.g. HCC, gastric carcinoma, testicular carcinoma, lung cancer and pancreatic cancer and some non-neoplastic disorders such as HC and hepatitis (Soresi *et al*., 2003). The association between serum AFP and HCC has been widely examined and described by a large number of groups. Regardless, its sensitivity and specificity for diagnosing HCC are variable, with figures ranging from 39 to 73% and 65 to 96%, respectively (Forner *et al*., 2009) depending on factors such as the specific assay used, the design of the study, the characteristics of the study population, and the designated cut-off level (Huo *et al.,* 2007). The chronic hepatic disease damages the liver and the resulting wound-healing process might lead to liver fibrosis and subsequent cirrhosis development. Fibrosis is the excessive deposition of extracellular matrix in the tissue as consequence of chronic liver damage. Thus, efforts to understand and attenuate fibrosis have direct clinical implications (Mas *et al*., 2009). Clinical management of chronic hepatitis C is dependent on the extent of liver fibrosis. Liver biopsy, the gold standard, is still recommended in the majority of patients (Strader *et al*., 2004). However, it is an invasive procedure responsible for severe complications in about 0.5% of cases. Sample variability is another limitation.

Table 3. Showing AFP in HCC and Control groups

Groups	Median	Minimum	Maximum	P value
HCC Patients	**30.6**	**0.8**	**2000**	**P<0.05**
Healthy Control	**3.0**	**1.5**	**4.0**	

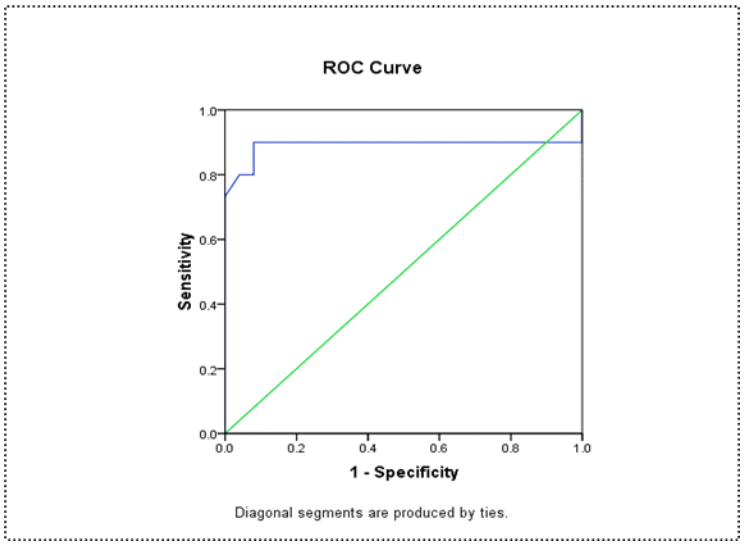

Fig. 4. ROC curve for AFP area under the curve (AUROC) 0.891, p <0.05

CONCLUSION

Hepatitis C virus (HCV) has been estimated by the World Health Organization (WHO) to infect 170 million patients worldwide, with the highest prevalence rate among Egyptians (14%-18%, approximately 10-fold greater than in the United States and Europe) it accounts for most chronic liver disease and HCC cases in Egypt. Hepatocellular carcinoma (HCC) is the third cause of death among those patients with malignant tumors. Chronic infection by hepatitis B or C virus (HBV and HCV) is the principal risk factors for HCC. Early diagnosis of HCC is the only hope for a cure, as most patients have the inoperable disease at the time of diagnosis. There was a correlation between APRI and FIB-4 fibrotic indices, Also Fibrotic Indices including APRI and FIB-4 are a non-invasive tool with diagnostic value and could differentiate between Hepatocellular, Cirrhotic patients.

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CONFLICT OF INTEREST

There is no conflict of interest.

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