Evaluation of biomarkers for the detection of hepatocellular carcinoma in patients with hepatitis C virus

Nadia Y.S. Morcos1, Ekram Z.I. Khafagi2, Mohamed S. Mogawer3, Mamdouh M. Ali2, Abeer H. Abdel-Halim2

1Department of Biochemistry, Faculty of Science, Ain Shams University; 2Department of Biochemistry, Division of Genetic Engineering and Biotechnology, National Research Centre; 3Department of Internal Medicine, Faculty of Medicine, Cairo University; Cairo, Egypt.

Abstract

Objective: Hepatocellular carcinoma (HCC) is the most frequent and severe complication of chronic liver diseases. It represents an important public health problem in Egypt, where up to 90% of HCC cases are attributable to hepatitis C virus (HCV) infection. The objective of the present study was to assess a panel of biomarkers that can significantly differentiate between HCC and non-HCC patients with chronic HCV infection (CHC), quantitatively.

Methods: A total of 75 adult male patients with CHC were divided into 3 main groups according to liver involvement: HCV without cirrhosis (CHC), patients with cirrhotic liver (LC), and HCC patients. Liver function, lipid profile, HBsAg, HCV antibodies, alpha-fetoprotein (AFP), insulin-like growth factor II (IGF-II), heparanase (HPSE), and lipid peroxidation were assayed.

Results: The AST/ALT ratio, AFP, and HPSE were significantly different in the HCC group with the optimum cut-off values as ≥ 1.92, 64.7 ng/ml and 5.6 U/ml, respectively. By using these cut-off values combined; 96% of HCC patients showed two abnormal markers, corresponding to only 29% of the LC group.

Conclusion: The use of combined HPSE, AFP and AST/ALT ratio cut-off values improved the positive predictive value for HCC from 79% to 96%.

Key words: Alpha-fetoprotein; AST/ALT ratio; Heparanase; Hepatocellular carcinoma; IGF-II

Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world, and the third most common cause of cancer-related death [1, 2], that kills more than 650,000 people around the world each year [3]. The burden of HCC has been increasing in Egypt with a doubling in the incidence rate in the past 10 years [4]. This has been mainly attributed to viral infection (hepatitis B and C virus), and dietary factors (e.g. aflatoxin B1) [5]. HCV causes HCC mainly through continuous inflammation and hepatocyte regeneration in the setting of chronic hepatitis and subsequent progression to cirrhosis, which is thought to lead to chromosomal damage and possibly to initiate hepatic carcinogenesis [6, 7].

HCC has great regional differences in the pathology and epidemiology, which is mainly influenced by the etiologies of the disease. The major established risk factors for HCC development are chronic viral hepatitis and liver cirrhosis [8, 9]. Early diagnosis of HCC is of great importance in order to offer the possibility of curative treatment [6, 10].

Surveillance programs have been conducted in many countries to detect HCC at an early stage. Alpha-fetoprotein (AFP) and ultrasonography are usually used as diagnostic tools [11].

Despite the scientific advances and the implementation of measures for early HCC detection in patients at risk, the overall survival of patients has not yet significantly improved during the last three decades. This is due to the late diagnosis of most HCC patients [12], when curative treatment is not possible, which is in turn due to extensive involvement of the liver, invasion of the hepatic or portal vein and/or the presence of metastasis [13]. Accordingly, the development of tumor markers that can detect HCC at earlier stages is essential. The functions of tumor markers include
prediction of prognosis or therapeutic response as well as diagnosis or screening of cancer [14]. As markers of HCC occurrence are still very scarce, and early detection of HCC increases the chance of treatment, consequently, the objective of the present study was to assess a panel of biomarkers that can significantly increase both the specificity and sensitivity to recognize HCC, quantitatively.

**Subjects and methods**

Seventy five adult male patients with chronic HCV infection were selected from the inpatients wards of the Internal Medicine Department at El-Kasr El-Ainy Hospital, and Liver Clinic and Liver Intensive Care in Nasser Institute, Cairo, Egypt. The study population was classified into the following groups according to liver involvement: 15 HCV without cirrhosis (CHC), 29 patients with cirrhotic liver (LC; 18 patients with Child Pugh A and B, and 11 patients with Child Pugh C according to Child Pugh classification [15]), and 31 patients with histologically proven HCC. Ten normal age-matched healthy adults were included as reference controls. A written consent was given by all participants, and the study was approved by the ethical committee at El-Kasr El-Ainy Hospital and National Research Centre, Cairo, Egypt.

The patients with other viral infections, diabetes mellitus, hypertension or bilharziasis were excluded from this study. Fasting blood samples were taken from all subjects, and the serum was separated by centrifugation at 3000 rpm and stored at –20°C until needed.

Each patient was subjected to the following:

- **Clinical assessment**: full medical history, the initial onset of the disease, its course and duration, ascites, bleeding tendency, hepatomegaly, lower limb edema, jaundice, and abdominal ultrasound. The severity of the underlying disease was assessed by the Child-Pugh score based on serum albumin, bilirubin, prothrombin time, the presence of ascites and encephalopathy [15].

- **Routine laboratory investigations**: urine and stool analysis, complete blood picture, serum creatinine, and liver function profile [serum bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), albumin (ALB), and prothrombin time].

- **Tumor markers**: heparanase enzyme (HPSE), IGF-II, AFP, oxidative stress as serum thiobarbituric reactive substances (TBARS), and nitric oxide (NO).

**Biochemical analysis**

**Determination of serum transaminases**

ALT and AST activities were determined in serum using a kit obtained from Biomerieux (Marcy l'Etoile, France). The produced keto acids (oxaloacetate or pyruvate) formed the corresponding colored hydrzones by reaction with 2,4-dinitrophenylhydrazine. The color was then measured at 505 nm.

**Determination of serum alkaline phosphatase activity**

Serum ALP activity was determined by colorimetric assay using a kit obtained from Biomerieux. In this procedure ALP acts upon phenylphosphate, the phenol liberated is measured in the presence of amino-4-antipyrine and potassium ferricyanide. The presence of sodium arsenate in the reagent stops the enzymatic reaction and simultaneously develops a blue chromophore, which is measured photometrically at 590 nm.

**Determination of serum creatinine level**

Determination of serum creatinine concentration was carried by Biomerieux kit, depending on the rate of formation of a colored complex between creatinine and alkaline picrate.

**Determination of serum bilirubin concentration**

Serum total bilirubin was determined using a kit obtained from Biomerieux. According to this method total bilirubin is determined by reaction with diazotized sulfanilic acid, in the presence of caffeine, with the final production of an azopigment, which is read at 578 nm.

**Determination of serum albumin concentration**

Serum ALB concentration was carried out again by a Biomerieux kit. Serum ALB binds selectively to the bromocresol green dye at pH 4.2. The increment of absorbance of resulting albumin-dye complex at 630 nm is proportional to the ALB concentration.

**Quantitative determination of alpha-fetoprotein in serum**

AFP concentration was determined using RADIM Diagnostics kit (Pomezia, RM, Italy). AFP is a glycoprotein with molecular weight of about 70 KDa and 3-4% carbohydrate content. It shares structural homology and amino-acid sequence with human serum albumin. This test is based on an immune-enzyme-metric assay (IEMA). Two different anti-AFP monoclonal antibodies are used, one adsorbed on the wells and the other conjugated to horseradish peroxidase (HRP). During the first incubation, the AFP in calibrators and samples is
bound to both monoclonals at once, by forming a ‘sandwich’. Following this incubation, the unbound material is removed by aspiration and washing. The residual enzyme activity found in the wells will thus be directly proportional to the AFP concentration in calibrators and samples and evidenced by incubating the solid phase with a chromogen solution (tetramethylbenzidine, TMB) in a substrate-buffer. Colorimetric reading will be performed by using a spectrophotometer at 450 nm.

**Quantitative measurement of insulin-like growth factor II in serum**

IGF-II level was determined using the DSL-10-2600 Active® Non-Extraction ELISA kit from Diagnostics Systems Laboratories (Webster, TX, USA) depending on the enzymatically amplified ‘two-step’ sandwich-type immunoassay. In the assay, standards, controls and unknown samples are incubated in microtitration wells, which have been coated with anti-IGF-II detection antibody labeled with the enzyme horseradish peroxidase (HRP). After a second incubation and washing step, the wells are incubated with the substrate TMB. An acidic stopping solution is then added and the degree of enzymatic turnover of the substrate is determined by dual wavelength absorbance measurement at 450 and 620 nm. The absorbance measured is directly proportional to the concentration of IGF-II present. A set of IGF-II standards is used to plot a standard curve of absorbance versus IGF-II concentration from which the IGF-II concentrations in the unknowns can be calculated.

**Determination of heparanase activity in serum**

Dye-binding assays have increased in popularity as an analytical technique for the measurement of glycosaminoglycan degrading enzymes. Determination of HPSE activity is depending on the method reported by Homer et al [16], heparan sulfate reacts with Stains-all dye (1-ethyl-2-[3-[1-ethyl-naphtho[1,2-d]thiazolin-2-ylidene]-2-methylpropenyl]naphtha[1,2-d]thiazolium bromide) to form a complex with an absorbance maximum at 478 nm. Increases in absorbance at the appropriate wavelength are directly proportional to the concentration of the heparan sulfate interacting with the dye. This phenomenon provided the basis for a sensitive spectrophotometric assay for the quantitative measurement of heparan sulfate-depolymerizing enzymes. The basic assay method was adapted for use in 96-well microtiter trays, thus enabling large numbers of assays to be carried out simultaneously.

**Assessment of lipid peroxidation**

TBARS as a marker of the lipid peroxidation were measured using the method of Yoshioka et al [17], based on the reaction of thiobarbituric acid with malondialdehyde, the second products of lipid, carbohydrate, protein, and DNA peroxidation as follows: a 0.5 ml sample was shaken with 2.5 ml of 20% TCA. To the mixture 1 ml of 0.67% thiobarbituric acid was added, shaken, and kept for 30 min in a boiling water bath followed by rapid cooling. Then 4 ml of n-butyl alcohol was added and shaken. The mixture was centrifuged at 3000g for 10 min. The resultant n-butyl alcohol layer was taken into a separate tube and the TBARS content was determined calorimetrically from the absorbance at 535 nm. 1,1,3,3-tetraethoxypropane was used as standard. Level of peroxidation products was expressed as the amount of TBARS in plasma (nmol/ml).

**Determination of nitric oxide**

Serum NO was determined according to the methods of Granger et al [18] based on that the NO production is detected in biological fluids via nitrite by using nitrite/nitrate assay using Roche Diagnostics kit (Mannheim, Germany). The nitrate present in the sample is reduced to nitrite by reduced nicotinamide adenine dinucleotide phosphate (NADPH) in the presence of the enzyme nitrate reductase. The nitrite formed reacts with sulfanilamide and N-(1-naphthyl)-ethylenediamine dihydrochloride to give a red-violet complex which measured at 550 nm. Level of NO in the serum was expressed as (µmol/ml).

**Statistical analysis**

Continuous variables are presented as mean values ± standard error, while categorical variables are presented as absolute number and frequencies. Associations between categorical variables were tested by the use of contingency tables and the calculation of chi-squared test. Differences between groups were evaluated by the calculation of Student's t-test and one-way ANOVA. Correlations between biochemical markers and other continuous variables were tested using the Spearman or the Pearson's correlation coefficients. Sensitivity, specificity, positive and negative predictive values, and diagnostic accuracy were calculated [19]. The predictive values of the studied parameters for the patients group was compared to control group data by ROC curve analysis, the data expressed as area under the curve (AUC) [20]. All reported p-values are based on two-sided tests and compared to a significance level of 5%.
Results
Activities of the liver enzymes (AST, ALT, and ALP) as well as ALB level in the studied groups are given in Table 1. Results revealed that AST activity was significantly higher ($p < 0.001$) in the HCC group as compared to the other groups (control by 403%, CHC by 161%, and LC by 94%), while the ALT activity was higher in HCC as compared to control group only (91%; $p < 0.05$). The albumin level decreased ($p < 0.001$) in all patients (CHC by 19%, LC by 53% and HCC by 49%) as compared to reference levels. Both groups of patients with LC and HCC had lower albumin levels when compared to the CHC group ($p < 0.001$; 42% and 38%, respectively), with no significant difference between LC and HCC groups. ALP activity was non-significantly different among all groups.

AST/ALT ratio showed significant increase ($p < 0.001$) in the HCC group as compared to the other groups (Table 1).

Evaluation of HCC markers and oxidative stress
The liver tumor markers AFP, IGF-II, HPSE and oxidative stress parameters (NO and TBARS) of the 85 subjects are given in Table 2. Results revealed that AFP level was significantly higher ($p < 0.001$) in HCC group as compared to the other groups (control by 4697%, CHC by 3484%, and LC by 1436%). The IGF-II level was not different between HCC and LC group, but was significantly lower in both groups when compared to control and CHC ones. HPSE activity was augmented with the severity of the liver damage, showing significant differences between the groups being highest in HCC, followed by LC then CHC, with the least activity in the control group.

Nitric oxide level was higher ($p < 0.001$) in both the HCC and LC groups as compared to either control or CHC groups. Although NO level in HCC was higher than that observed in the LC group, the difference did not reach to the significant level. Lipid peroxidation as indicated by TBARS level in the patients groups was higher than that of control, meanwhile, the highest level was observed in the HCC group, which was higher ($p < 0.001$) than both the CHC and LC groups by 33% and 23%, respectively.

Table 1. Demographic and clinical data of the studied groups (one way ANOVA)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Chronic hepatitis C (CHC)</th>
<th>Liver cirrhosis (LC)</th>
<th>Hepatocellular carcinoma (HCC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>29.4 ± 2</td>
<td>43 ± 2.3</td>
<td>53 ± 1.6</td>
<td>55.1 ± 1.5</td>
</tr>
<tr>
<td>Child Pugh classification</td>
<td>-</td>
<td>-</td>
<td>12 C, 16 B, 1 A</td>
<td>18 C, 11 B, 2 A</td>
</tr>
<tr>
<td>Meld score</td>
<td>6.4 ± 0.2</td>
<td>8.1 ± 2.1</td>
<td>19 ± 1.4</td>
<td>18.4 ± 1.7</td>
</tr>
<tr>
<td>ASL (U/l)</td>
<td>27 ± 2</td>
<td>52 ± 7</td>
<td>70 ± 7</td>
<td>136 ± 19</td>
</tr>
<tr>
<td>ALT (U/l)</td>
<td>29 ± 2</td>
<td>42 ± 6</td>
<td>43 ± 5</td>
<td>55 ± 8</td>
</tr>
<tr>
<td>ALP (U/l)</td>
<td>81 ± 7</td>
<td>101 ± 13</td>
<td>112 ± 13</td>
<td>136 ± 17</td>
</tr>
<tr>
<td>ALB (mg/dl)</td>
<td>4.8 ± 0.1</td>
<td>3.9 ± 0.2</td>
<td>2.3 ± 0.1</td>
<td>2.4 ± 0.1</td>
</tr>
<tr>
<td>AST/ALT ratio</td>
<td>0.95 ± 0.07</td>
<td>1.29 ± 0.15</td>
<td>1.75 ± 0.15</td>
<td>2.65 ± 0.25</td>
</tr>
</tbody>
</table>

Data are represented as mean ± SEM. Significantly different from: *control group, †CHC group, and ‡LC group ($p < 0.001$).

Table 2. Comparative liver tumor markers data in the different studied groups and the control group (one way ANOVA)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Chronic hepatitis C (CHC)</th>
<th>Liver cirrhosis (LC)</th>
<th>Hepatocellular carcinoma (HCC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFP (ng/ml)</td>
<td>6.5 ± 0.7</td>
<td>8.7 ± 1.</td>
<td>20.3 ± 2.9</td>
<td>311.8 ±42.7</td>
</tr>
<tr>
<td>IGF-II (ng/ml)</td>
<td>1146 ± 90</td>
<td>493 ± 58†</td>
<td>242 ± 28.9†</td>
<td>268 ± 15.8†</td>
</tr>
<tr>
<td>HPSE (U/ml)</td>
<td>2.94 ± 0.07</td>
<td>3.71 ± 0.12‡</td>
<td>4.35 ± 0.08.9‡</td>
<td>5.67 ± 0.13.3‡</td>
</tr>
<tr>
<td>NO (µmol/ml)</td>
<td>9.36 ± 0.77</td>
<td>14.17 ± 0.9</td>
<td>28.6 ± 1.1.9</td>
<td>31.42 ± 1.3.9</td>
</tr>
<tr>
<td>TBARS (nmol/ml)</td>
<td>3.64 ± 0.26</td>
<td>5.72 ± 0.33‡</td>
<td>6.2 ±0.36</td>
<td>7.59 ± 0.3</td>
</tr>
</tbody>
</table>

Data are represented as mean ± SEM. Significantly different from: *control group, †CHC group, and ‡LC group ($p < 0.001$).
Further statistical analyses were carried out in order to evaluate the supremacy of HPSE, AFP and AST/ALT as diagnostic tools to differentiate between HCC and the other groups.

ROC curve analyses were applied for the HCC group with the other groups combined (Non-HCC), and for HCC vs liver cirrhosis (LC). The negative (NPV) and positive (PPV) predictive values for the observed cut-off values were tabulated to determine their significance. This was done using cross-tabulation and applying Fischer’s exact test ($\chi^2$) and Pearson correlation analyses. Results are presented as figures and tables showing the area under curve (AUC), significance (p), cut-off value, NPV and PPV. ROC curves for these markers are shown in Figures 1 and 2.

Table 3 shows the chosen cut-off values for HPSE, AFP and AST/ALT ratio as markers for HCC. The first cut-off value for HPSE was 4.75 U/mL with sensitivity 81% and specificity 100%. The second one was 5.65 U/mL with sensitivity 100% and specificity 64%. The normal AFP cut-off value as given in the literature [21,22] is 20 ng/mL, this value showed 74.5% sensitivity and 68.6% specificity. On the other hand, the optimum cut-off value from the present study was found to be 64.7 ng/mL - with 100% sensitivity and 61% specificity. The best observed cut-off value for the AST/ALT ratio was 1.92 with 83% sensitivity and 71% specificity.

Figures 3-7 show the cross-tabulations for the calculated cut-off values of AFP, HPSE and AST/ALT ratio, using Fischer’s exact test for the NPV and PPV. For AFP ≥ 20 ng/ml it gave a higher PPV (79% vs 61%), while using the AFP < 64.7 ng/ml gave 100% NPV.

Figures 5 and 6 show that the value 4.7 U/ml of HPSE gave a higher PPV (93.5% vs 58%), while using the 5.6 U/ml gave 100% NPV. 1.92 cut-off values for AST/ALT ratio gave 74% PPV for HCC patients (Fig.7).

Table 3. Observed sensitivity and specificity for the chosen cut-off values

<table>
<thead>
<tr>
<th>Marker</th>
<th>Cut-off</th>
<th>% Sensitivity</th>
<th>% Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPSE U/mL</td>
<td>4.75</td>
<td>81</td>
<td>100</td>
</tr>
<tr>
<td>5.65 U/mL</td>
<td>64.7</td>
<td>100</td>
<td>61</td>
</tr>
<tr>
<td>AFP ng/mL</td>
<td>20</td>
<td>74.5</td>
<td>68.6</td>
</tr>
<tr>
<td>64.7 ng/mL</td>
<td>1.92</td>
<td>83</td>
<td>71</td>
</tr>
</tbody>
</table>

Figure 1. ROC curves for HPSE, AFP and AST/ALT in HCC patients vs the other groups combined (non-HCC).

Figure 2. ROC curve for HPSE, AFP and AST/ALT ratio in HCC patients vs LC.

Figure 3. Columns representing the percentage of patients with high risk of developing HCC by using AFP level ≥ 20 ng/ml.
Figure 4. Columns representing the percentage of patients with no risk of developing HCC by using AFP level < 64.7 ng/ml.

Figure 5. Columns representing the percentage of patients with high risk of developing HCC by using HPSE activity ≥ 4.7 U/ml.

Figure 6. Columns representing the percentage of patients with no risk of developing HCC by using HPSE activity < 5.6 U/ml.

Figure 7. Columns representing the percentage of patients with high risk of developing HCC by using AST/ALT ratio ≥ 1.92.

Figure 8. Percent of patients with abnormal levels of either one or two markers in the different groups.

Figure 9. Percent of patients with abnormal levels of more than one marker in the different groups.

The cross tabulations using two and three markers are illustrated in Figures 8 and 9. Figure 8 shows that by using HPSE and AFP combined with each other resulted in 79% of HCC patients had two abnormal markers and 21% had one abnormal marker. In cirrhotic patients 21% had two abnormal markers and 29% had one abnormal marker. Figure 9 shows that by using HPSE, AFP and AST/ALT ratio 96% of HCC patients had two abnormal markers corresponding to 29% of LC patients.

Discussion
The ideal study design would be to compare cirrhosis that develops into HCC and cirrhosis that does not develop into HCC in the follow-up of the disease, but such samples were very scarce as the follow-up among cirrhotic patients would be extremely long and difficult. Therefore, we compared the levels of these markers, single or combined, in three groups of patients all suffering from chronic HCV infection; these groups represented cirrhosis-free/HCC-free (CHC), HCC-free/cirrhosis (LC), and HCC patients.
The results revealed that the percent of patients with abnormal levels of more than one marker in HCC group was increased from 79% to 96% by using three markers combined. The increase was significantly correlated to the degree of liver injury, AFP, and HPSE activity, even after controlling for age.

From the ROC curve analysis the optimum AST/ALT cut-off value was 1.92 where, 26% of the HCC patients were negatively diagnosed, and 24% of the LC patients were positively diagnosed with HCC. Pohl et al [23] and Giannini et al [24] reported comparable observations. However, other studies found that the AST/ALT had no correlation with the presence of HCC [25, 26].

Although oxidative stress markers (NO and TBARS) were significantly correlated to the degree of liver injury, AFP, HPSE, and AST/ALT ratio, their role as diagnostic markers for HCC was incompetent and cannot discriminate between HCC and the other studied CHC groups. AFP level was significantly higher in HCC group compared to all groups. It was found that the AFP cut-off value of 64.7 ng/ml is better than the 20 ng/ml; meanwhile it cannot be considered as an ideal marker for HCC. These results were matched with Tsai et al [27] who found that the median level of serum AFP in patients with cirrhosis alone was statistically higher than that of healthy controls and that the optimal cut-off value selected was 50 ng/ml for HCC patients. Similarly, Snowberger et al [28] reported that AFP was elevated in 20% without HCC, but exceeded 100 ng/ml in only 3%, and that the overall accuracy of AFP was poor regardless of the cut-off. In a recent study by Zinkin et al [22], their mean results for AFP in cirrhotic patients was 35 ng/ml (range, 1-730) versus 19390 ng/ml (range, 1.3-207160) in HCC.

IGF-II level was significantly lower in both LC and HCC groups when compared to control and CHC groups, yet was not statistically different among the HCC and LC groups. IGF-II did not show any selective power as an HCC marker, as shown from both the ROC curve and the discriminating analyses. Hayakawa et al [29] explained these declines on the basis that IGF-II level increases temporarily in early HCC and then decreases gradually in the process of HCC development. According to Fan et al [30], this is because once the pre-cancerous liver cells are transformed into malignant; they acquire autogrowth ability and become independent from IGF-II to maintain their malignant proliferation. According to Yu et al [31], Reeves et al [32], and Chen et al [33], levels of IGF-II are low at birth and increase with age through puberty. After puberty, the levels decline slowly with age. These observations verify our results. Thus, we are inclined to believe that IGF-II is not a reliable marker for HCC; this may possibly be due to the advanced age and HCC stage of the patients included in our study. Consequently, we recommend that the age factor should be taken into consideration when assessing the consistency of IGF-II as an HCC marker.

HPSE is a heparan sulfate (HS) degrading endoglycosidase participating in extracellular matrix degradation and remodeling [34]. There are few studies on the potency of HPSE as a marker for HCC [35]. Previous publications clearly link heparanase expression to the process of tumorigenesis in a wide number of cancers, which were reviewed by Zhang et al [36], and Dong and Wu [37].

In the present study HPSE is considered as a sensitive marker of HCC, where its activity began to increase from the beginning of inflammation as in CHC patients, then in cirrhotic patients, reaching its highest activity in HCC patients. It was revealed that only 6% of HCC patients were false negative and 31% of LC patients were false positive at cut-off value 4.7 U/ml. Meanwhile 42% of HCC patients were not diagnosed at cut-off value 5.6 U/ml. Nadir et al [34] and Chen et al [35, 38] reported comparable observations.

From the present study we have concluded that AST/ALT ratio could be used as a marker for HCC; nonetheless its performance might improve when combined with other markers. Lipid peroxidation marker was not able to discriminate between HCC and the other studied CHC groups. AFP cannot be considered as an ideal marker for HCC. IGF-II is not a reliable marker for HCC and the age factor should be taken into consideration when assessing the consistency of IGF-II as an HCC marker. From the foregoing results we can conclude that, the percent of patients with abnormal levels of more than one marker in HCC group was increased from 79% to 96% by using three markers combined.
References


