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# Evaluation of Serum Total Bile Acids in the Diagnosis of Hepatocellular Carcinoma

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## ABSTRACT

Fasting total serum bile acids (FSBA) concentrations were measured in 140 cases of chronic liver diseases including 50 patients with liver cirrhosis, 40 patients with chronic hepatitis and 50 patients with hepatocellular carcinoma (HCC). FSBA concentrations were significantly higher in patients with HCC than those with chronic hepatitis and cirrhosis and both were significantly higher than the controls ($p < 0.0001$). FSBA concentrations were not correlated to the size of the tumor, the Child Pugh grades, the histopathologic grades and most of the liver function tests. Using the receiver operative characteristic (ROC) and the differential positive rate (DPR) analysis, 30 μmol/l was the optimal cut-off value that differentiates patients with HCC from those with cirrhosis. At this level, the sensitivity, the specificity and the diagnostic accuracy were 42%, 98% and 70% respectively. AFP was found to be significantly increased in patients with HCC than those with chronic hepatitis and cirrhosis. The best cut-off value of AFP was 100 ng/ml, at which the sensitivity, the specificity and diagnostic accuracy were 52%, 96% and 74% respectively. The simultaneous determination of AFP and total bile acids raised the sensitivity of the test to 76%. The area under the ROC curve for AFP and total bile acids was 0.764 and 0.744 respectively, the difference is non-significant. In conclusion, both AFP and total bile acids are good markers for HCC and their simultaneous determination may improve the detection of HCC in cirrhotic patients negative for AFP.

***Key Words:*** *Fasting total serum bile acids - AFP - Hepatocellular carcinoma.*

## INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common malignant neoplasms. It is associated closely with cirrhosis [8,14,16] and its prognosis is very poor because the diagnosis is generally late, when the disease is so advanced that any effective treatment is precluded. Therefore, early detection is an important factor in the management of this type of cancer.

Bile acids are organic anions, mainly synthesized in the liver and they are taken up and excreted in a manner generally similar to bilirubin. Bile acids offer two advantages over bilirubin as a test of liver function. First, their flux, which is determined by pool size and frequency of entrohepatic cycling, is a hundred-fold greater than that of bilirubin, potentially magnifying abnormalities associated with milder degrees of liver injury. Second, bile acid concentrations are not affected by abnormalities as that observed in bilirubin transport [9]. The sensitivity of serum bile acids in patients with chronic liver disease is better than serum albumin and prothrombin time because the value depends not only on hepatic injury, but also on excretory function and portal systemic shunting [7]. Serum bile acids have been measured both during fasting and two hours postoprandially, as well as after oral and intravenous administration. Postprandial bile acids and intravenous and oral bile acid tolerance tests do not provide better information than do fasting levels alone [5,6]. Fasting total serum bile acids (FSBA), were assayed in patients with hepatocellular carcinoma, patients with chronic liver disease and control subjects [1]. The authors reported a significant increase of the level of FSBA in the HCC group than those patients with chronic liver disease ($p < 0.01$) and the control group ($p < 0.001$).

The present work is a trial to evaluate the diagnostic accuracy of FSBA in diagnosis of HCC in a cirrhotic population and to determine a cut-off value differentiating cirrhotics from patients with HCC.

## PATIENTS AND METHODS

Between January 1998 and July 1999, one hundred and forty patients with chronic liver diseases were selected from the outpatient clinics and inpatient departments of the Tropical Medicine Department, Al-Hussein Hospital, Al-Azhar University and the Medical Oncology Department National Cancer Institute, Cairo University. The study population was classified into the following patient groups:

Group 1: Includes 50 patients with histologically proven hepatocellular carcinoma (HCC) being 39 males and 11 females with a mean age of 57.69±8.96 years.

Group 2: Includes 50 patients with cirrhosis. They were 27 males and 23 females with a mean age of 50.68±10.95 years.

Group 3: Includes 40 patients with chronic active hepatitis, 26 chronic active hepatitis C virus and 14 chronic active hepatitis B virus. They were 33 males and 7 females with a mean age of 45.8±12.3 years.

Group 4: Includes 35 normal healthy adults as controls, including 22 males and 13 females with mean age of 38.37±11.46 years.

Serum samples were taken from fasting patient subjects and controls and stored at -80°C until being used. Table (1) summarizes all the characteristics of patients and controls.

*Each patient was subjected to the following:*

- Clinical assessment including history taking and physical examination.
- Routine laboratory investigations including urine and stool analysis, complete blood picture and serum creatinine.
- Liver function profile (serum bilirubin, AST, ALT, alkaline phosphatase, albumin and prothrombin time).
- The severity of underlying disease was assessed by Child-Pugh score based on serum albumin, bilirubin, prothrombin time, the presence of ascites and encephalopathy [10].
- Fasting total bile acids (FSBA) were estimated after a 12 hours overnight fasting in all subjects, patients and controls, by the enzymatic, calorimetric method [13] using a commercially available kit by Randox laboratories ltd, United kingdom, BT 294 QY. The results were expressed in µmol/l.

*Statistical analysis:*

All data were expressed as mean value ± standard deviation. The relationship between continuous variables were analyzed by Spearman's product moment correlation coefficient. Mean values of continuous variables were compared using ANOVA or *t*-test. The significance level was set at *p*-value less than 0.05. The Mann-Whitny U-test was used to compare the medians of continuous variables.

Sensitivity, specificity, positive and negative predictive values, positive and negative likelihood ratios and diagnostic accuracy were calculated according to the following formula [11].

Sensitivity = a / (a+c).

Specificity = d / (b+d).

Accuracy = (a+d) / (a+b+c+d).

Positive predictive value = a / (a+b).

Negative predictive value = d / (c+d).

Positive likelihood ratio = Sensitivity (1-specificity).

Negative likelihood ratio = (1-sensitivity) / specificity.

Where; a = true positive cases, b = false positive cases, c = false negative cases, d = true negative cases.

Receiver operating characteristic (ROC) curves were constructed by calculating the sensitivities and specificities of AFP and total bile acids at several cut-off points. Also, the differential positive rate plot (DPR) (true positive rat - false positive rate) was constructed by calculating DPR at different decision levels. The highest point of the plot is at the optimum cut-off or decision level [15]. The differences in diagnostic accuracy between the marker test were measured by Mc Nemar's test.

## RESULTS

*Serum total bile acids:*

The mean value of serum total bile acids in patients with HCC was significantly higher (39.77±30.63 µmol/l) than that found in patients with cirrhosis (20.17±14.92 µmol/l) or with chronic hepatitis (15.46±8.66 µmol/l) and in control subjects (6.94±3.75 µmol/l, $p < 0.0001$). Also, significant differences were found between controls and patients cirrhosis and between controls and chronic hepatitis (Table 2).

Fig. (2) illustrates the individual values for serum total bile acids in patients and controls. It is obvious that there are 21 patients with HCC, 1 patient with cirrhosis and 3 with chronic hepatitis had serum total bile acids above the cut-off value while healthy individuals have shown no elevation above the cut-off value.

*Serum total bile aids and AFP as diagnostic markers for HCC evaluated by ROC curve:*

Based on the significant increase of both AFP and total bile acids in patients with HCC than the other patients and controls, an attempt was made to differentiate HCC from cirrhosis by these two markers. ROC curves for both markers are shown in Fig. (1). The calculated area under the ROC curve is 0.764 for AFP and 0.744 for total bile acids. The sensitivity of each marker was determined at several specificity levels. The corresponding sensitivities and actual cut-off points producing Fig. (1) are given in Table (4). The optimal cut-off values selected by the ROC curves were 100 ng/ml and 30 μmol/ml for AFP and total bile acids, respectively.

According to the ROC analysis, the optimal cut-off level for AFP was 100 ng/ml. The sensitivity and specificity were 96% and 52%, respectively, with a diagnostic accuracy of 74%, a positive likelihood ratio of 13 and a negative likelihood ratio of 0.5 (Table 3). The optimal cut-off value of serum total bile acids was 30 μmol/l, which gave a specificity of 98% at a sensitivity level of 40%. The calculated diagnostic accuracy and positive and negative likelihood ratios were 70%, 21 and 0.59 respectively. The area under the ROC curve of AFP (0.764) was slightly higher than that of total bile acids (0.744), the difference was statistically insignificant. When both AFP and total bile acids were determined in parallel, 12 (50%) of 24 patients with HCC negative for AFP (less than 100 ng/ml) could be diagnosed. The sensitivity, the specificity and diagnostic accuracy were 76%, 88% and 100% respectively with positive and negative likelihood ratios of > 76 and 0.24 respectively. The diagnostic accuracy of using both AFP and total bile acids as markers was significantly higher than using AFP alone ($p <$ 0.001).

*Correlation between AFP and serum total bile acids:*

Using the Spearman's correlation coefficient, no significant correlation could be found between AFP and serum total bile acids. The regression equation correlating the two variables is $y = -6.2229x + 51.891$ ($r = -0.188$, $p > 0.05$). This indicates that AFP and serum total bile acids are independent variables and so their simultaneous determination may increase the sensitivity of the test.

*Serum total bile acids and the tumor size:*

Serum total bile acids were not correlated to tumor size. In patients with tumor size > 5 cm, total bile acids concentration was (36.95±30.81 μmol/l) ad (44.36±30.59 μmol/l) in patients with tumor size < 5 cm. The difference was statistically insignificant ($p = 0.412$) (Table 3).

Table (1): Main characteristics of the three patient groups; HCC, cirrhostics, chronic hepatitis and controls. HCC: Hepatocellular carcinoma, CH: Chronic hepatitis, SD: Standard deviation.

| Group | Males | Females | Age | | |
|---|---|---|---|---|---|
| | | | Mean ± SD | Median | Range |
| HCC | 39 (78%) | 11 (22%) | 57.69±8.996 | 55 | 40-70 |
| Cirrhosis | 27 (54%) | 23 (46%) | 50.68±10.95 | 52.5 | 21-75 |
| CH | 33 (82.5%) | 7 (17.5%) | 45.8±12.3 | 48 | 17-80 |
| Controls | 22 (62.8%) | 13 (37.2%) | 38.37±11.46 | 36 | 21-61 |

Table (2): Mean ± standard deviation of total serum bile acids in patients with hepatocellular carcinoma (HCC), patients with cirrhosis and patients with chronic hepatitis (CH) as well as in controls.

| Group | Number of patients | Total bile acids (µmol/l) | F-value | *p* |
|---|---|---|---|---|
| HCC | 50 | 39.77±30.63 | | |
| Cirrhosis | 50 | 20.17±14.92 | 29.5 | < 0.0001 |
| CH | 40 | 15.54±8.66 | | |
| Controls | 30 | 6.94±3.75 | | |

Table (3): Mean ± standard deviation of serum total bile acids levels in patients with HCC and their relation to the tumor size, Child-Pugh grades and histopathologic grades as compared by the Student *t*-test.

| | | Total bile acids (µmol/l) | *p* |
|---|---|---|---|
| Tumor size | > 5 cm | 36.95±30.81 | |
| | < 5 cm | 44.36±30.5 | 0.412 |
| Child Pugh grade | A | 48.35±31.92 | |
| | B or C | 33.45±29.13 | 0.1 |
| Histopathologic grade | I | 48.68±22.43 | 0.191 (I&II) |
| | II | 32.19±24.9 | 0.93 (I&III) |
| | III | 49.92±42.36 | 0.18 (II&II) |

Table (4): Sensitivity, specificty, diagnostic accuracy, positive and negative predictive values (PV), positive and negative likelihood ratios (LR) and differential positive rates (DPR) of AFP and total bile acids at different cut-off levels and the simultaneous use of both markers in the diangosis of HCC in cirrhotic patients. A:AFP . 100 ng/ml, B: total bile acids > 30 µmol/l.

| Cut-off value of AFP (ng/ml) | Sensitivity % | Specificity % | Diagnostic accuracy % | Positive PV % | Ngative PV % | Positive LR | Negative LR | DPR |
|---|---|---|---|---|---|---|---|---|
| 13 | 80 | 52 | 66 | 62.5 | 72.2 | 1.66 | 0.38 | 32 |
| 15 | 78 | 58 | 68 | 65 | 72.5 | 1.85 | 0.38 | 36 |
| 20 | 72 | 68 | 70 | 69.2 | 70.8 | 2.25 | 0.41 | 40 |
| 30 | 66 | 74 | 70 | 71.7 | 68.5 | 2.53 | 0.46 | 40 |
| 40 | 60 | 78 | 69 | 73.2 | 66.1 | 2.72 | 0.51 | 38 |
| 60 | 56 | 86 | 71 | 80 | 66.15 | 4 | 0.51 | 42 |
| 100 | 52 | 96 | 74 | 92.9 | 66.6 | 13 | 0.5 | 48 |
| 160 | 46 | 98 | 72 | 95.8 | 64.5 | 23 | 0.55 | 44 |
| 200 | 42 | 98 | 70 | 95.4 | 62.8 | 21 | 0.59 | 40 |

| Cut-off value of bile acids µmol/l | Sensitivity | Specificity | Accuracy | Positive PV | Ngative PV | Positive LR | Negative LR | DPR |
|---|---|---|---|---|---|---|---|---|
| 16 | 80 | 52 | 66 | 62.5 | 72.2 | 1.66 | 0.38 | 32 |
| 17 | 74 | 60 | 67 | 64.9 | 69.8 | 1.85 | 0.43 | 34 |
| 20 | 68 | 68 | 68 | 68 | 68 | 2.13 | 0.47 | 36 |
| 23 | 52 | 78 | 65 | 70.3 | 61.9 | 2.36 | 0.62 | 30 |
| 28 | 44 | 92 | 68 | 75.9 | 63.2 | 5.5 | 0.61 | 36 |
| 30 | 42 | 98 | 70 | 95.5 | 62.8 | 21 | 0.59 | 40 |
| 35 | 38 | 98 | 68 | 100 | 61.2 | 19 | 0.63 | 36 |

| Marke r | Sensitivity % | Specificity % | Diagnostic accuracy % | Positive PV % | Ngative PV % | Positive LR | Negative LR | DPR % |
|---|---|---|---|---|---|---|---|---|
| A | 52 | 96 | 74 | 92.9 | 66.6 | 13 | 0.5 | 48 |
| B | 42 | 98 | 70 | 95.5 | 62.8 | 21 | 0.59 | 40 |
| A or B | 76 | 100 | 88 | 100 | 80.7 | > 76 | 0.24 | 76 |

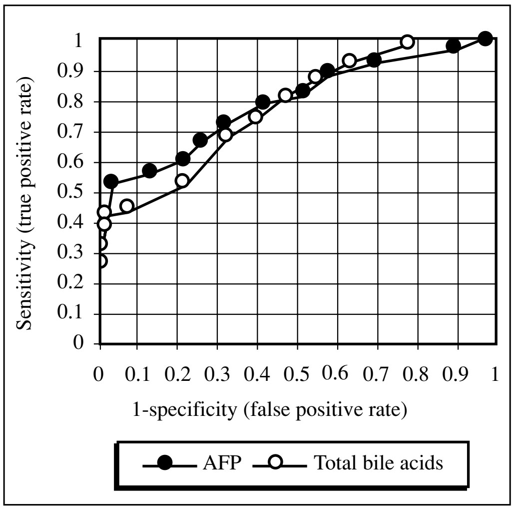


Fig. (1): Receiver operating characteristic (ROC) curves of AFP and total bile acids plotted for the diagnosis of HCC in cirrhotics. The area under the curve is 0.764 for AFP and 0.744 for total bile acids. The difference is not significant.

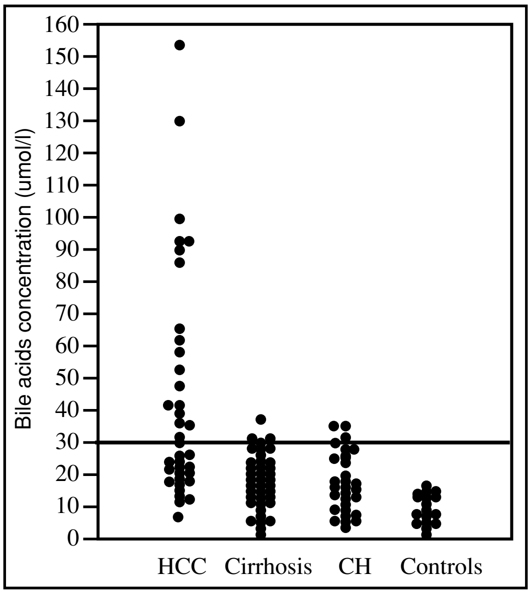


Fig. (2): Dot diagram of total bile acids in patients with HCC, cirrhosis, CH an controls. The horizontal line represent the cut-off value (30 µmol1/1). HCC: hepatocellular carcinoma; CH: chronic hepatitis.

## DISCUSSION

In the present study, the increased levels of total bile acids in serum of patients suffering from liver cancer, come in agreement with the work of Changbumrung et al. [3] and Abdel-Rahim et al. [1]. Total bile acids, in the current study, were significantly higher in patients with HCC than those with cirrhosis, chronic hepatitis and control subjects ($p < 0.0001$). The elevation of total bile acids concentration in patients with chronic liver disease is thought to results from a reduced hepatic clearance and/or from portosystemic shunting [7]. Tam et al. [13] reported high levels of total bile acids in patients with chronic liver disease; they found that the concentration of total bile acids is related to the severity of liver disease being higher in the acute, exacerbated or decompensated stage. Agha and Zuberi [2] found that postprandial serum bile acids were high in patients with chronic liver disease and that the frequency of abnormal bile acids was greater than that of abnormal standard liver function tests. Fujiwara et al. [4] reported the detection of unusual bile acids in sera of patients with HCC. Changbumrung et al. [3] found unconjugated primary and secondary bile acids in sera of patients with cholangiocarcinoma and HCC whereas these bile acids were not detected in any of the controls.

For clinical decision making the selected cut-off value of a laboratory test should provide the best diagnostic performance for either ruling out or ruling in a particular disease. The receiver operating characteristic curve (ROC) is a graphic method which can be used to determine this optimal cut-off level. In addition, it is precise and valid measure of diagnostic accuracy [11]. The differential positive rate curve (DPR) is also useful in determining the cut-off level.

*Serum total bile acids and the Child-Pugh grades of HCC:*

Serum total bile acids were not correlated to the Child-Pugh grades. In patients with Child

A, total bile acids showed a non-significant increase (48.35±31.92 µmol/l) over Child grades B and C (33.45±29.13 µmol/l, $p = 0.1$) (Table 3).

*Serum total bile acids and the histopathologic grades of HCC:*

With respect to tumor grades, no statistical significant differences were found between patients with grade I (48.68±22.43 µmol/l), grade II (32.19±24.9 µmol/l) and grade III (49.92± 42.36 µmol/l).

The "*p*" values for comparisons between grades I and II, I and III as well as II and III were 0.191, 0.93 and 0.18 respectively (Table 3).

*Serum total bile acids and liver function tests:*

In patients with HCC, total bile acids was slightly positively correlated to serum AST ($r = 0.2649$; $p < 0.05$), negatively correlated to alkaline phosphatase ($r = -0.3106$; $p < 0.01$) and prothrombin concentration ($r = -0.354$; $p < 0.001$) but not correlated to albumin ($r = -0.1698$; $p > 0.05$), ALT ($r = 0.0386$; $p > 0.05$), nor total bilirubin ($r = -0.1467$; *p* total bilirubin ($r = 0.041$, 0.124, 0.129, 0.178 and 0.036 respectively $p > 0.05$ in all cases).

Where the maximum point of the curve is that of choice [15].

Using ROC and DPR analysis, the optimal cut-off level of FSBA and AFP were 30 µmol/l. At this level, FSBA showed a low sensitivity (42%), a high specificity (98%) and a moderate diagnostic accuracy (70%). The area under the ROC curve was 0.744 and 0.764 for FSBA and AFP, respectively. The areas were between 0.7 and 0.9 indicating that both markers are useful for diagnostic purposes. Determination of AFP and total bile acids in parallel increased the diagnostic accuracy to 88% (Table 4). Although each test alone may not have sufficient sensitivity, the simultaneous use of both tests may be highly discriminatory in the detection of HCC. The sensitivity of AFP alone was 52% and that of total bile acids alone was 42% while the sensitivity of both tests when carried out simultaneously increased to 76%. The positive predictive value and the positive likelihood ratios increased from 92.9 and 13 for AFP alone to 100% and > 76 for both tests, respectively.

Total serum bile acids showed no significant correlation with AFP, tumor size, Child-Pugh grades or the histopathologic grades. FSBA levels were positively correlated to serum AST and negatively correlated to alkaline phosphatase which indicates that total bile acids are related to liver necrosis.

The high concentrations of total bile acids in HCC patients in the current and in a previous study [1] as well as the presence of unusual bile acids reported by Changbumrung et al. [3] suggest a role for serum total bile acids measurements for early detection and screening of HCC.

In conclusion, this study revealed that the addition of an assay for FSBA to AFP gives a significant improvement in detection of HCC in patients with cirrhosis. The optimal cut-off value for AFP in the diagnosis of HCC was 100 ng/ml and that of total bile acids was 30 µmol/l. Furthermore, the test for FSBA should be used as an adjunctive tool in the detection of HCC in cirrhotics negative fro AFP.

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