Squamous Cell Carcinoma Antigen as a Tumor Marker in Patients with Hepatocellular Carcinoma

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Abstract

Background: Hepatocellular carcinoma (HCC) is considered the fifth most common cancer in the world. Owing to its increased incidence in the last decade and the expected further increase in the next 2 decades, HCC is arousing great interest. HCC commonly develops on cirrhotic livers and therefore, surveillance programs have been suggested to identify early HCC, at a stage suitable for surgical or interventional therapy and has a better clinical outcome. The only serologic marker used in clinical practice is α-fetoprotein (α-FP), but its sensitivity is poor. Hence, the investigation of new markers is required.

Aim of the Study: To assess the clinical utility of squamous cell carcinoma antigen (SCCA) as a non invasive marker in the early diagnosis of HCC and whether the association of α-FP and SCCA could improve the diagnostic power.

Subjects and Methods: This study is conducted on 65 newly diagnosed hepatic focal lesion cases from those attending the Tropical Medicine Department, Cairo University Hospitals (Group I) as well as 20 age and sex matched healthy control subjects (Group II). Group I was further subdivided into Ia (49 HCC proved untreated patients) and Ib (16 patients with Cirrhosis only) according to their histopathologic findings. All patients were subjected to full history taking, clinical examination, laboratory investigations (including liver function test, hepatitis markers, α-FP and SCCA serum levels), triphasic abdominal CT and pathological examination.

Results: Group I included 42 males (64.7%) and 23 females (35.3%) with ages ranging between 42-70 years (60.7± 11.28), of them 16 patients had HBV (24.6%), 37 patients had HCV (56.9%) and 12 patients (18.4%) had mixed HBV and HCV infection. Group I was further subdivided into group Ia which included 49 HCC proved patients and group Ib (16 patients with Cirrhosis only) according to their histopathologic findings. All patients were subjected to full history taking, clinical examination, laboratory investigations (including liver function test, hepatitis markers, α-FP and SCCA serum levels), triphasic abdominal CT and pathological examination.

Conclusion: Combined use of α-FP and SCCA in the screening of patients with hepatic focal lesions may increase the chance of diagnosis of HCC patients.

Key Words: α-FP – SCCA – HCC – Cirrhosis – Tumor marker.

Introduction

HEPATOCELLULAR carcinoma (HCC) is the 5th most common cancer worldwide and the third most common cause of cancer-related death. In Egypt, there was an annual significant rise of HCC ranging from 3.6% in 1992 to 5.3% in 1995 [1]. HCC is a well-known complication of chronic hepatitis [2,3]. Asymptomatic patients diagnosed as HCC through screening programs are more likely to be candidates for curative treatment and have improved short- and medium-term survival [4,5]. Therefore, surveillance programs aimed at detecting early stage HCC have been recommended by the European Association for the Study of the Liver (EASL) as well as the Italian Association for the Study of the Liver (AISF). These programs are based on the use of ultrasound tomography and α-fetoprotein (α-FP). The reliability of imaging techniques has greatly improved in the last years but such diagnostic procedures are expensive and subject to interpretation.

On the other hand, as the only diagnostic serologic test currently available in clinical practice, serum α-FP had been shown to be associated with HCC since 1963 [6], unfortunately it is also elevated in a wide variety of non-hepatic malignancies [7,8].
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and benign hepatic conditions [9,10]. α-FP has too low sensitivity and specificity; based on receiving operating characteristic (ROC) curve analysis, its sensitivity reaches only 60% [8]. Thus, searching another tumor marker, that together with AFP could improve the diagnostic utility of the later, seemed to be justified.

SCCA is a component of the high molecular weight serine protease inhibitors named serpins [11]. SCCA is physiologically expressed in the squamous epithelia and increased levels have been detected in several epithelial cancers such as those of the head, neck, cervix and lung. It has been reported that the squamous cell carcinoma antigen (SCCA) is over expressed in HCC tissues [10]. A different expression of SCCA in HCC and peritumoral tissue has recently been documented, together with a higher concentration of this antigen in the serum of HCC patients than cirrhotic patients [12,13].

The aim of this study is to investigate whether SCCA serum levels represent a useful tool for HCC diagnosis and whether SCCA is a better marker than α-FP and finally whether, in association with α-FP, SCCA could improves the diagnostic power.

Subjects and Methods

The study was conducted on newly diagnosed 65 patients with underlying hepatic focal lesion attending the Tropical Medicine Department, Cairo University between May 2006 and March 2007 (group I). Twenty healthy age and sex matched individuals served as the control group (group II). Informed consents were obtained from all participants in the study.

Patients included in the study were subjected to thorough history taking, clinical examination, laboratory evaluation (including liver function test, hepatitis markers, α-FP and SCCA serum levels) and ultrasound examination. All patients diagnosed to have hepatic focal lesion on ultrasound examination were further subjected to triphasic CT abdomen and guided biopsy from the hepatic focal lesion.

Analytic procedures:

After overnight fasting, Blood samples were collected from all patients from antecubital vein by venipuncture. Whole blood samples remained chilled for approximately 30 minutes. Blood samples were centrifuged at 3000 rpm and plasma was separated and stored at -20 °C until time of assay. Serum AST, ALT and albumin were done on the Hitachi 917 instrument by routine analytical methods [14,15] (Roche diagnostics GmbH, D.68298 Mannheim). Hepatitis markers were assessed by EIA using the AxSYM autoanalyser [16] (Abbott laboratories Diagnostic Division Max-Planck-Ring2 65205 Germany). Serum α-FP measured by (IRMA): A solid phase two site sequential chemiluminescent immunoradiometric assay on the Immulite Autoanalyzer (DPC diagnostic products corporation, Los Angeles) [17]. Serum SCCA measured by (EIA): A solid phase non-competitive direct sandwich enzyme-immunoassay for the quantitative measurement of SCCA in serum (CanAg diagnostics AB, SE-414 55 Gothenburg,Sweeden) [18].

Abdominal US:

It was done for all patients using convex probe of 3.5MHz Toshiba (ECCOCCE) SSA-340 A, to document the presence of hepatic focal lesions with their descriptive data (Fig. 1) and looks for signs of chronic liver disease and portal hypertension [19].

Triphasic abdominal CT:

Patients with a focal lesion on US were further investigated with triphasic CT scan. The typical specific enhancement pattern for the diagnosis of HCC is the arterial uptake (Fig. 2), followed by venous washout in the delayed portal/venous phase [20]. Lymph nodes were measured at the time of assessment.

For all patients with hepatic focal lesions, histopathology was made using US-guided fine needle biopsy (an 18 -gauge Tru-Cut needle) (Fig. 3). All biopsies results were examined by the same histopathologist. Patients with cholangiocarcinoma, hepatoblastoma and liver metastases were excluded.

Group I was further subdivided into group Ia included 49 HCC proved patients and Ib included 16 patients with hepatic cirrhosis, according to their histopathologic findings.

Statistical methods:

Results obtained were analyzed; data were summarized as mean and standard deviation and compared using t-test in comparing between two groups and analysis of variance (ANOVA) in comparing more than two groups. Significant results were followed-up by Bonferroni post hoc test, non-Gaussian data were summarized as medians with interquartile range (25 th-75 th percentile) and they were then log-transformed to confirm normal distribution. Quantitative data were compared using spearman rho correlation coefficient (rs). In all
tests, *p* value was considered significant if <0.05 and highly significant if <0.001. The optimal cutoff for different analyte were calculated by constructing a receiver operating characteristic (ROC) curve and odds ratio were calculated for each parameter at the selected cutoff value.

**Fig. (1):** Ultrasound picture: Hypoechoic focal lesion (HCC).

**Fig. (2):** Arterial phase of spiral CT: Full enhancement of HCC with a feeding vessel (arrow).

**Fig. (3):** Histopathology of HCC (grade II).

**Results**

Group I included 42 males (64.7%) and 23 females (35.3%) with ages ranging between 42-70 years (60.7±11.28), of them 16 had HBV (24.6%), 37 patients had HCV (56.9%) and 12 patients (18.4%) had mixed HBV and HCV infection. Group I was further subdivided into group Ia which included 49 HCC proved patients and group Ib which included 16 patients with regeneration nodules (cirrhosis only) according to their histopathologic findings. Group II (control) included 20 age and sex matched healthy subjects. They were 12 males (60%) and 8 females (40%). Their ages ranged between 38-64 years (53.7±5.80). All of them had no history of HBV and HCV infection as proved by laboratory investigations.

When using the ROC (receiver operator curve), to determine the best specificity and sensitivity of both analyses, at the cutoff value of 40ng/ml, the specificity and sensitivity of α-FP were 100% and 67.2% respectively. While, at the cutoff value of 2.55ug/L, the specificity and sensitivity of SCCA were 100% and 61.2% respectively. The area under the curve (AUC) for α-FP was 0.859 with a confidence interval ranging from 0.776-0.942 while that of SCCA was 0.788 with a confidence interval ranging from 0.689-0.886.

Using different cut off values as reported in different literatures, serum α-FP at a cutoff of 200ng/mL, the sensitivity was 35% and the specificity was 100% while at a cutoff >400ng/mL, the sensitivity decreased to 7.6%. While, at the cutoff of serum SCCA 1.2 (the kit cutoff) the sensitivity was 65.2% and the specificity was 36.7%.

When combined sensitivity of them was calculated at the best-chosen cutoff values, sensitivity improved to 87.7% with specificity of 100%.

**Table (1):** The clinical and laboratory results of studied cases.

<table>
<thead>
<tr>
<th></th>
<th>Group Ia (n=49)</th>
<th>Group Ib (n=16)</th>
<th>Group II</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cirrhosis etiology:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCV</td>
<td>27</td>
<td>10</td>
<td>No</td>
</tr>
<tr>
<td>HBV</td>
<td>10</td>
<td>6</td>
<td>No</td>
</tr>
<tr>
<td>HCV &amp; HBV</td>
<td>12</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td><strong>Child-Pugh score:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>10</td>
<td>5</td>
<td>–</td>
</tr>
<tr>
<td>B</td>
<td>19</td>
<td>2</td>
<td>–</td>
</tr>
<tr>
<td>C</td>
<td>20</td>
<td>9</td>
<td>–</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>38.27±6.32</td>
<td>42.6±4.28</td>
<td>14.9±2.11</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>55.45±24.21</td>
<td>59.73±3.67</td>
<td>23.85±5.70</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>2.44±0.1</td>
<td>3.42±0.23</td>
<td>3.9±1.02</td>
</tr>
</tbody>
</table>
Table (2): The level of α-FP in group Ia, group Ib and group II.

<table>
<thead>
<tr>
<th></th>
<th>Group Ia (n=49)</th>
<th>Group Ib (n=16)</th>
<th>Group II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median</td>
<td>290.0</td>
<td>67.50</td>
<td>4.40</td>
</tr>
<tr>
<td>25th-75th percentile</td>
<td>9.30-370.00</td>
<td>5.70-220.50</td>
<td>2.00-6.00</td>
</tr>
</tbody>
</table>

p value between group Ia & Ib = 0.001, between group Ia & II <0.0005 and between Ib & II = 0.035.

Table (3): The level of SCCA in group Ia, group Ib and group II.

<table>
<thead>
<tr>
<th></th>
<th>Group Ia (n=49)</th>
<th>Group Ib (n=16)</th>
<th>Group II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median</td>
<td>2.84</td>
<td>1.52</td>
<td>0.70</td>
</tr>
<tr>
<td>25th-75th percentile</td>
<td>0.30-2.10</td>
<td>0.20-0.90</td>
<td>0.00-0.60</td>
</tr>
</tbody>
</table>

p value between group Ia & Ib = 0.001, between group Ia & II <0.0005 and between Ib & II = 0.043.

Table (4): Combined sensitivity and specificity for α-FP and SCCAg.

<table>
<thead>
<tr>
<th>SCCA</th>
<th>Cirrhosis (&lt;40ng/ml)</th>
<th>HCC (&gt;40 ng/ml)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cirrhosis (&lt;2.55ug/L)</td>
<td>22</td>
<td>16</td>
<td>38</td>
</tr>
<tr>
<td>HCC (&gt;2.55ug/L)</td>
<td>10</td>
<td>17</td>
<td>27</td>
</tr>
<tr>
<td>Total</td>
<td>32</td>
<td>33</td>
<td>65</td>
</tr>
</tbody>
</table>

+ve α-FP/+ve SCCA = 17/49, +ve α-FP/-ve SCCA = 16/49, -ve α-FP/+ve SCCA = 10, Total = 43/49 = 87.7%

Discussion

Hepatocellular carcinoma (HCC) had become a major health problem worldwide. A major improvement of treatment and survival has been achieved in patients with HCC at the initial stage [18-19]. HCC most often develops in patients with chronic liver disease [21,22]. Therefore, surveillance programs have been suggested for patients with liver cirrhosis to identify early HCC, at a stage when it remains suitable for surgical or ablative therapy and has a better clinical outcome. The only serologic marker used in clinical practice is α-fetoprotein, but its sensitivity is poor [9,12]. Specific serologic markers have not yet been identified for screening of high risk patients. Squamous cell carcinoma antigen is a component of the high molecular weight serine protease inhibitors named serpins [12]. A different expression of SCCA in HCC and peritumoral tissue has recently been documented, together with a higher concentration of this antigen in the serum of HCC patients than cirrhotic patients [11-13].

The sensitivity and specificity of α-FP for the detection of HCC has been reported from 17 to 76% and 80 to 100%, respectively [23-26]. Arrieta et al. [27] found that an α-FP value of 200 and 400ng/mL has a sensitivity of 36.3% and 20.2%, respectively and specificity of 100% in both groups, similar results were found in this study, at the cutoff of serum α-FP 200ng/mL the sensitivity was 35% and the specificity was 100% while at a cutoff >400ng/mL. The sensitivity decreased to 7.6%.

Being of low sensitivity α-FP is not an ideal marker for HCC diagnosis or screening. On the other hand, SCCA has a better sensitivity; in our results at the cutoff of serum SCCA 1.2 (the kit cutoff) the sensitivity was 65.2% and the specificity was 36.7%. Similar results were found by Giannelli et al. [12] the cutoff of serum SCCA 0.3 (the kit cutoff) the sensitivity was 84.2% and the specificity was 48.9%.

When combined sensitivity of both markers, calculated at the best-chosen cutoff values (SCCA 2.55ug/L and α-FP40 ng/ml) sensitivity improved to 87.7% with specificity of 100%. Matching results were found by by Giannelli et al. [12], although a different cut off was used, combining both markers increases the sensitivity. The used cutoff values were 0.3ng/ml for SCCA and 20IU/ml for α-FP, there was a 90.83% correct diagnosis rate among the HCC patients (109/120), with 44.44% (40/90) true negatives among the cirrhotic patients. The positive predictive value was 68.55% (109/159). Although there remained a low specificity, the number of correct HCC serologic diagnoses increased to 55.05% (60/109).

These results seem encouraging, although the partial overlap of the SCCA reference limits affects the ability to detect HCC among cirrhotic patients. This could be explained by the relatively low specificity of the SCCA antigen.
Nevertheless, because of its high sensitivity, the diagnostic discrimination between HCC and liver cirrhosis is improved in those cases where α-FP is not elevated. Regarding the false positives among the cirrhotic patients, it is also possible that the altered immune response commonly observed in cirrhotic patients may affect the specificity of the ELISA kit used for SCCA detection. Although it is not yet clear why a squamous epithelial antigen such as SCCA is strongly expressed in HCC and the functions for both α-FP and SCCA remain unknown, we cannot rule out the possibility that biologic characteristics of the malignancy may affect the expression level of such an antigen [12].

In conclusion: The SCCA antigen represents a useful marker for HCC detection. In association with α-FP, it significantly increases the reliability of serologic diagnosis of this cancer. However, more studies are needed to realize the potential of HCC serologic diagnosis.

References


