Detection of Epithelial Membrane Antigen and Determination of its Diagnostic Performance in Patients with Hepatocellular Carcinoma

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ABSTRACT

Background: Hepatocellular carcinoma (HCC) is considered the sixth most common cancer in the world. Epithelial membrane antigen (EMA) is a glycoprotein which is associated with the tumor formation and has an important role in metastasis. During the process of tumor cells invasion and transformation, the changes in the glycolization of EMA effect on the tumor biological behavior. The aim of the present study is the identification of EMA in serum samples in patients with HCC, detection of EMA in serum samples in patients with HCC and determination of detection rate of EMA in serum samples in patients with HCC.

Methods: Total number of 100 patients with HCC and 35 healthy controls were recruited. Clinical and laboratory data were measured in patients and controls. Serum EMA rate was assessed using western blotting and ELISA.

Results: HCC group had higher liver enzymes, bilirubin and INR and a lower to mean platelet counts and albumin levels than controls. HCC group was significantly higher (P < 0.001) in AFP level than that of Control group. Also, there was a highly significant difference (P < 0.001) between the level of EMA in HCC group and control group. Epithelial membrane antigen was identified in serum samples of hepatocellular carcinoma by using monoclonal antibody (EMA mAb) and western blot technique at 130 kDa.

Conclusions: Since, the EMA was detected by ELISA with a sensitivity of 81% and a specificity of 100%, EMA can be used in HCC diagnosis.

Keywords: HCC, Liver Cancer, AFP, Epithelial Membrane Antigen

1. Introduction

Hepatocellular carcinoma (HCC) is considered the sixth most common cancer in the world. It is one of the main causes of mortality in cirrhotic patients. In Egypt, the number of patients which are diagnosed with hepatocellular carcinoma increases every year. The hepatocellular carcinoma prevalence is high in the area of Nile Delta and is more common in males than females, particularly in chronic hepatitis C patients (Abdel-Wahab et al., 2007). Chronic infection with the hepatitis B or hepatitis C is the most important causes of hepatocellular carcinoma...
Hepatocellular carcinoma symptoms are similar to liver cirrhosis, such as ascites, fever, fatigue, weight loss, and epigastric pain. More than 40% of patients with HCC has no symptoms which makes the early diagnosis of hepatocellular carcinoma difficult (Schacherer et al., 2007). The detection of any liver mass can be done by various diagnostic tests like magnetic resonance imaging, computed tomography and an increased level of serum markers. Although, it is difficult to distinguish between the hepatocellular carcinoma and other conditions and the ultrasound (US) has a low sensitivity for the detection of hepatocellular carcinoma. Ultrasound is very safe, but it is limited by air and bone and depends on the operator. Computed tomography (CT) is faster and more generic, but the ionizing radiation is considered a drawback.

Although alpha fetoprotein is specific and is considered the most common biomarker used for the diagnosis of HCC, but it is not suitable for the HCC surveillance because it has low specificity, sensitivity and positive predictive value and leading to the diagnosis delay.

Epithelial membrane antigen (EMA) is from a series of mucins and it is also named MUC1 (ten Berge et al., 2001). EMA is a specific component of the human milk fat globule membrane. EMA was found in various neoplastic epithelial cells (Sloane and Ormerod, 1981). EMA is a glycoprotein which is associated with the tumor formation and has an important role in metastasis. During the process of tumor cells invasion and transformation, the changes in the glycolization of EMA effect on the tumor biological behavior. (Byrd and Bresalier, 2004).

The aim of the present study is the identification of EMA in serum samples in patients with HCC, detection of EMA in serum samples in patients with HCC and determination of detection rate of EMA in serum samples in patients with HCC.

2. Material and methods

2.1. Sample collections

A total of 135 serum samples were included in this study, of which 35 of them from healthy individuals as negative controls, negative for anti-HCV antibodies, and 100 of them from patients with HCC associated with HCV (positive for anti-HCV antibody) were collected from Mansoura University hospitals, Mansoura, Egypt. For all the subjects, clinical examination and ultrasonography of the abdomen were done. The diagnosis of hepatocellular carcinoma was based on alpha fetoprotein >400 U/L, the presence of liver focal lesion(s) detected by ultrasound and confirmed by computed tomography and/or magnetic resonance. The diagnosis of HCC was carried out according to the American Association For The Study Liver Diseases (AASCD) practice guide lines (Bruix and Sherman, 2011). Blood samples were collected from all patients by vein-puncture at the time of diagnosis. A part of the blood was treated with EDTA-K3 for complete blood count (CBC). The second portion was treated with citrate solution for prothrombin INR (International Normalized Ratio). Serum was immediately separated by centrifugation, aliquoted, stored at -20 °C until used at the time of the assay.

Liver function tests, CBC and AFP were done. Liver function tests were measured on an automated biochemistry analyzer (Hitachi 902; Roche Diagnostics). Complete blood count was performed on KX-21 Symex automated hematology analyzer (Sysmex corporation, kobe, Japan). AFP was performed by chemiluminescence, with Immulite AFP (1000) kit (Diagnostic Products Corporation; Los Angeles, CA, USA). In addition to 35 serum samples from healthy individuals served as normal controls.
2.2. *Estimation of protein content*

Lowry’s method is the most widely used and accepted method for accurate determination of protein level within 0.01–1.0 mg/mL. The method is a combination of biuret reaction and folin-ciocalteu reaction. Protein binds to copper in alkaline medium and produces Cu+ then Cu+ catalysis oxidation of aromatic amino acid by reducing phosphomolybdotungstate to heteropolymolybdanum blue which predominantly depends upon tyrosine and tryptophan and other residues in protein. The sensitivity of the reaction increase (within 20%) by adding Folin reagent (Hess et al., 1978).

2.3. *Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)*

Polyacrylamide, is a matrix formed from acrylamide and bis-acrylamide with different pore sizes to produce gels with different separation properties. To separate proteins, based on their molecular weight, the tertiary structure is destroyed by reducing agent the protein to a linear molecule. SDS binds fairly uniformly to the linear proteins, the charge of the protein is now approximately proportional to its molecular weight. The SDS-treated proteins move toward the cathode at different rates depending on their molecular weight. The polyacrylamide chain is initiated by TEMED, which induces free radical formation from ammonium persulphate (APS). The stacking gel at pH 6.8 has a low concentration of acrylamide and the running gel a higher concentration capable of retarding the movement of the proteins.

2.4. *Immunoblotting Technique (Western Blot)*

Western blotting refers to the transfer of protein from a gel to a nitrocellulose membrane then detection of protein on the surface of the nitrocellulose membrane. Western blotting can produce qualitative analysis. After electrophoresis, the separated proteins are transferred onto nitrocellulose membrane. Nitrocellulose membrane is blocked to prevent any nonspecific binding of antibodies to the surface of the Nitrocellulose membrane. Transferred protein is detected with a specific antibody, then substrate is added to the enzyme to produce a chromogenic product on Nitrocellulose membrane.

2.5. *Purification of the EMA antigen*

Preparative gel electrophoresis capable of yielding high milligram to gram quantities of proteins recovered from the corresponding analytical gels. Analytical SDS-PAGE can be adapted for preparative purposes by increasing gel thickness.

2.6. *Detection of EMA antigen using ELISA*

ELISA combines the specificity of antibodies with the sensitivity of enzyme by using antibodies coupled to an easily-assayed enzyme. ELISA can provide useful measurements of antigen concentrations.

2.7. *Statistical Analysis*

All statistical analyses were done by a statistical software package (SPSS 15.0 for Microsoft Windows, SPSS Inc.). Descriptive results were expressed as mean ± SD, SE and range or number (percentage) of patients with a condition.
Differences in continuous variables were assessed using student t-test or ANOVA and $\chi^2$ test for categorical variables. All tests were two-tailed and statistical significance assessed at the 0.05 level.

**Results**

In the present study serum samples from patients with hepatocellular carcinoma and healthy individuals as negative controls were tested by 12 % one dimensional (SDS-PAGE) under reducing conditions. The coomassie blue stained separated polypeptides have ranged from 215 kDa to 18.3 kDa (Figure 1).

![Figure 1: Coomassie blue stained SDS-PAGE showing the polypeptide pattern of serum samples from normal individuals and patients with hepatocellular carcinoma. Lanes 1-4: Sera of 4 healthy (negative controls). Lanes 5-8: Sera of 4 patients with hepatocellular carcinoma.](image)

The separated proteins were transferred to nitrocellulose paper. EMA mAb and anti-mouse IgG alkaline phosphatase and BCIP/NBT substrate were used for identification of EMA in sera of hepatocellular carcinoma. An intense sharp band of EMA was identified in sera of HCC at 130 kDa but no reaction with serum samples of normal individuals was observed (Figure 2).
Figure 2: Immunoblots of EMA in sera of patients with HCC. EMA mAb and anti-mouse IgG alkaline phosphatase and BCIP/NBT substrate were used for identification of EMA in sera of hepatocellular carcinoma. Lanes 1-4: serum of 4 healthy individuals (negative controls). Lanes 5-8: serum of 4 patients with HCC.

To determine the molecular weight of the reactive epitope for epithelial membrane mAb in serum samples of HCC patients, liner calibration represented a relation between the log molecular weight (log MW) of standard proteins mixture and their flow rates (R_f) on SDS-PAGE was constructed (Table 1). The flow rate of the reactive band was calculated and its log molecular weight was determined from the liner calibration. The molecular size of the reactive band was 130 kDa in serum samples of HCC patients (Figure 3).

Table 1: Relative motilities (R_f) values of unknown antigen and molecular weights of standard proteins mixture

<table>
<thead>
<tr>
<th>Molecular weight (MW)</th>
<th>Log molecular weight (Log MW)</th>
<th>Relative motilities (R_f)</th>
</tr>
</thead>
<tbody>
<tr>
<td>215.0 (myosin)</td>
<td>2.33</td>
<td>0.07</td>
</tr>
<tr>
<td>120.0 (phosphorylase B)</td>
<td>2.07</td>
<td>0.23</td>
</tr>
<tr>
<td>84.0 (bovine serum albumin)</td>
<td>1.92</td>
<td>0.33</td>
</tr>
<tr>
<td>60.0 (ovalbumin)</td>
<td>1.77</td>
<td>0.46</td>
</tr>
<tr>
<td>39.2 (carbonic anhydrase)</td>
<td>1.59</td>
<td>0.63</td>
</tr>
<tr>
<td>28.0 (trypsin inhibitor)</td>
<td>1.44</td>
<td>0.77</td>
</tr>
<tr>
<td>18.3 (lysozyme)</td>
<td>1.26</td>
<td>0.96</td>
</tr>
<tr>
<td>EMA (130 kDa)</td>
<td>2.11</td>
<td>0.21</td>
</tr>
</tbody>
</table>

Figure 3: Linear calibration represents R_f values of the unknown antigen in comparison with standard proteins mixture

The 130 kDa EMA was purified from sera of patients with hepatocellular carcinoma using electro-elution technique. The protein content of EMA was 2500 μg/ml. The purity of EMA antigen was evaluated by coomassie blue stained SDS-PAGE. A single band was identified at 130 kDa (Figure 4).
Figure 4: SDS-PAGE of the purified EMA. Lane 1: Serum sample of normal individual (negative control). Lane 2: Serum sample of patient with HCC (positive control). Lane 3: Trichloroacetic acid (TCA) precipitate fraction of purified 130 kDa. Lane 4: Trichloroacetic acid (TCA) supernatant fraction.

The epithelial membrane monoclonal antibody identified an intense sharp band corresponding to the purified 130 kDa EMA. It was observed in serum sample of patient with HCC (positive control) and TCA reconstituted precipitate but not with TCA supernatant soluble fraction (Figure 5).

Figure 5: Immunoblot analysis of the 130 kDa purified EMA. Purified EMA was resolved in 12 % SDS-PAGE and electroblotted onto NC. Lane 1: Serum sample from normal individual (negative control). Lane 2: Serum sample from patient with HCC (positive control). Lane 3: Trichloroacetic acid (TCA) precipitate fraction of purified 130 kDa. Lane 4: Trichloroacetic acid (TCA) supernatant fraction.

Serial concentrations of purified 130 kDa EMA were tested to establish a standard curve using ELISA. The dose curve was plotted using millimeter paper reporting the optical densities of EMA on Y-axis and the corresponding concentration of purified EMA (µg/ml) on X-axis. The concentration of each unknown sample was determined by interpolation from the dose-response curve (Figure 6).
A total of 135 serum samples were included in this study, of which 35 (26%) of them from healthy individuals as negative controls, negative for anti-HCV antibodies, and 100 (74%) of them from patients with positive for anti-HCV antibody. Patients with HCC were included 73 males and 27 females, aged 39-79 years, and mean age ± SD, 56.7±8.1 and healthy individuals were included 26 males and 9 females, and aged 30-66 years with mean age ± SD = 53.8±10.3 (Table 2).

**Table 2:** Demographic data of HCC patients and healthy individuals:

<table>
<thead>
<tr>
<th>Variables</th>
<th>Healthy</th>
<th>HCC</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number</td>
<td>35</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>26 (74%)</td>
<td>73 (73%)</td>
<td>-</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>9 (26%)</td>
<td>27 (27%)</td>
<td>-</td>
</tr>
<tr>
<td>Age (yrs), mean ± SD</td>
<td>53.8±10.3</td>
<td>56.7±8.1</td>
<td>&gt; 0.05</td>
</tr>
</tbody>
</table>

Patients with HCC had higher level of AST, ALT, alkaline phosphatase, total bilirubin and INR levels and a lower level of platelet counts and albumin. There was significant difference in the levels of evaluated blood markers (AST, ALT, total bilirubin, platelet count and albumin) between studied groups (Table 3).

**Table 3:** Laboratory data of HCC patients and healthy individuals:

**Figure 6:** Standard curve of purified EMA using ELISA

AFP (alpha fetoprotein) is used as a marker for diagnosis of hepatocellular carcinoma. AFP levels were measured in the sample set at cutoff value of 400 U/L. There was a significant difference in the level of AFP in healthy individuals and patients with HCC (Table 3).
Immunoblot is not suitable for routine determination of EMA. This could prove difficult for laboratories with limited resources. Epithelial membrane mAb was used as a probe in ELISA to quantified EMA in serum samples. The detection of EMA using ELISA is a simple, rapid and quantitative assay technique. To quantified EMA, monoclonal antibody specific for EMA was used. Serum samples were coated onto the surface of wells of ELISA plat, residual sticky sites were blocked by adding BSA. EMA mAb and anti-mouse IgG alkaline phosphatase and BCIP/NBT substrate were used for detection of EMA in sera of hepatocellular carcinoma.

The expression of EMA in serum was quantified by using the ELISA technique for individuals with different pathology of the liver. The cutoff level of EMA above or below which the tested sample is considered positive or negative was calculated as the mean + 3 standard deviation, the cut-off level of ELISA technique was set at OD = 0.26. From dose curve the cut-off level (OD = 0.26 at 405 nm) was set at 1.88 µg/ml, and serum samples from 16 patients with HCC showed concentration above the cut off level (Figures 7).

![Figure 7: The cutoff level of EMA was 1.88µg/ml.](image)

According to the obtained dose curve, the level of EMA was quantified in selected 100 patients with HCC against sera collected from 35 healthy individuals as a negative control. The mean ± S.E (µg/ml) of EMA concentration in HCC patients were 6.74±0.87 µg/ml. However, those in normal individual samples were 0.93±0.08 µg/ml. Overall significance of difference among the HCC patients and normal individual samples was determined by t-test for EMA (µg/ml). There are highly significant difference (P < 0.001) between the level of EMA in HCC patients and

<table>
<thead>
<tr>
<th>Variables</th>
<th>Healthy (n=35)</th>
<th>HCC (n=100)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (Aspartate aminotransferase) (U/L)</td>
<td>26.8±5.6</td>
<td>94.4±59.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ALT (Alanine aminotransferase) (U/L)</td>
<td>27.3±6.2</td>
<td>62.6±45.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ALP (alkaline phosphatase) (U/L)</td>
<td>67.8±27.0</td>
<td>222.4±137.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Bilirubin (mg/dl)</td>
<td>0.54±0.13</td>
<td>2.7±1.80</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>43.5±3.7</td>
<td>30.1±5.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Platelet count (×10^9/L)</td>
<td>251.0±37.2</td>
<td>121.6±64.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>INR (International Normalized Ratio)</td>
<td>1.0±0.10</td>
<td>1.5±0.40</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>AFP (alpha fetoprotein) (U/L)</td>
<td>5.7±0.40</td>
<td>3895±1041</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
healthy individuals. The median of serum EMA (µg/ml) in HCC patients and normal individuals were 2.8 and 0.8; respectively (Figure 8).

Figure 8: The mean level of EMA in HCC patients and healthy individuals using ELISA. Cut-off value of EMA is up to 1.88 µg/ml

Serum samples of 100 patients with hepatocellular carcinoma were tested by ELISA for the detection of EMA against sera collected from 35 healthy individuals, as a negative control. The EMA was detected in 81/100 (81%) of serum samples from HCC patients and was found 19/100 (19%) were negative for EMA. While, all 35 serum samples from healthy individuals were negative for EMA with detection rate (0.0%) (Table 4).

Table 4: Detection rate of EMA:

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>EMA using ELISA + Ve</th>
<th>EMA using ELISA - Ve</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Healthy individuals</td>
<td>35</td>
<td>0%</td>
<td>35%</td>
<td>100%</td>
</tr>
<tr>
<td>HCC patients</td>
<td>100</td>
<td>81%</td>
<td>19%</td>
<td>19%</td>
</tr>
</tbody>
</table>

The diagnostic value is assessed by ROC curve. Using ROC curves, we assessed the diagnostic accuracy of 130-kDa EMA represented among patients with HCC compared to healthy individuals. The AUC of EMA was 0.977 and the best cut-off was 1.88 µg/ml (Figure 9) (Table 5).

Table 5: Diagnostic performance of 130-kDa EMA:

<table>
<thead>
<tr>
<th>Subjects</th>
<th>No</th>
<th>130-kDa EMA using ELISA</th>
<th>Detection rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>+ ve</td>
<td>- ve</td>
</tr>
<tr>
<td>Healthy individuals</td>
<td>35</td>
<td>0</td>
<td>35</td>
</tr>
</tbody>
</table>

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Patients with HCC | 100 | 81 | 19 | 81.0 %

**Figure 9:** The ROC curve of serum 130-kDa EMA. The AUC was 0.97.

**Discussion**

HCC is one of the most common cancer and Most HCC cases occur in Africa and Asia. HCV appears to be the most causative agent for HCC development due to DNA damages in hepatocytes which will lead to increasing gene alteration (Shiraha et al., 2013). Hepatocellular carcinoma increased in Egypt in the last 10 years. This due to several etiological agents such as viral hepatitis and other toxins such as aflatoxin. Other factors such as cigarette smoking, pesticides and endemic infections (schistosomiasis) (Anwar et al., 2008). Imaging techniques steady evolution and play an important role in the diagnosis of HCC (Befeler and Di Bisceglie, 2002). Despite evolution in imaging technology, there is still a need for new markers to distinguish between HCC and liver cirrhosis. AFP associated with viral hepatitis, liver cirrhosis and liver tumors (Abu El Makarem, 2012). AFP is not useful for the early diagnosis of liver tumors and is not particularly for HCC surveillance because, even though 80% of the HCC patients have AFP level exceeding normal levels (França et al., 2004). Because the limitations of AFP, the search for more sensitive markers for HCC has continued. EMA (MUC1) (ten Berge et al., 2001) is present in a variety of secretory and non-secretory epithelium (Murakata et al., 2000).

In this study, patients with HCC had higher liver enzymes, bilirubin and INR and a lower to mean platelet counts and albumin levels. AFP used as marker for HCC diagnosis. AFP cutoff value was set at 400 U/L. There was a statistically significant difference in the level of AFP in healthy individuals and patients with HCC. (Gomaa et al., 2009) used AFP level at 400-500 ng/mL as HCC diagnostic marker from patients with liver cirrhosis. This cut-off value are not common in smaller tumors (< 5 cm) and only 30% of HCC patients were higher than 100 ng/mL (França et al., 2004). (Lau and Lai, 2008) reported that AFP is elevated in 65% of HCC patients than 10 ng/ml. Patients with high AFP should be evaluated with imaging techniques. For the diagnosis of HCC, AFP had a sensitivity of 39%-65%, a specificity of 76%-94%. The sensitivity and specificity of Golgi protein 73 were 69%
and 75% (Gomaa et al., 2009). (Shang et al., 2012) reported that Osteopontin is a glycoprotein produced by several different cell types, particularly bone and epithelial cells, and highly expressed in various cancers, including HCC. For differentiating early HCC from cirrhosis, the plasma osteopontin at 91 ng/mL cutoff has 75% sensitivity of and 62% specificity. And these values are lower than the diagnostic performances of EMA.

ELISA is simple, rapid, sensitivity and specificity diagnostic methods for detect antigen (Grauballe et al., 1981). In this study, the expression of EMA in serum was quantified by using the ELISA technique for individuals with different pathology of the liver. The EMA cutoff level was calculated as the EMA mean of 16 samples from normal individuals + 3 SD. The cut-off level of ELISA technique was set at OD = 0.26. From dose curve the cut-off level (OD = 0.26 at 405 nm) was set at 1.88 µg/ml, and serum samples from 16 patients with HCC showed concentration above the cut off level. Accordingly, and due to most serum samples from HCC patients showed concentration above the cutoff level, the estimation of serum EMA can be considered as a diagnostic test for HCC. (Rabassa et al., 2006) detected MUC1 using ELISA based on two anti MUC1 core protein mAb (BC2 and BC3). MUC1 cut off was at 4 U/ml.

In this study, EMA was quantified in 100 HCC patients using ELISA against sera collected from 35 healthy individuals as a negative control. The mean ± S.E (µg/ml) of EMA concentration in HCC patients were 6.74±0.87 µg/ml. However, those in normal individual samples were 0.93±0.08 µg/ml. Overall significance of difference among the HCC patients and normal individual samples was determined by ANOVA test for EMA (µg/ml). (Hendrick et al., 1986) estimated the mean level in healthy individuals at 500 ± 125 ng/ml. In this study, level of EMA were tested by ELISA in serum of 100 HCC patients and 35 healthy individuals. The EMA was detected in 81/100 (81%) of serum samples from HCC patients and was found 19/100 (19%) were negative for EMA. While, all 35 serum samples from healthy individuals were negative for EMA with detection rate (0.0%). In this study, there was a weak correlation (r = 0.232; P = 0.02) between level of EMA concentration (µg/ml) with INR in patients with HCC. Moreover, there was a weak correlation (r = - 0.453; P = 0.0001) between level of EMA concentration (µg/ml) with serum albumin (g/L). In contrast, there was no correlation between EMA levels (µg/ml) with other laboratory parameters. The EMA concentration increased in liver fibrosis patients more than the level in sera of healthy individuals. This is due to very low EMA expression on healthy adenocytes. The abnormal MUC1 produced new protein which identified by immune system as tumor antigens (Attallah et al., 2011).

In this study, HCC samples and healthy individuals were resolved using SDS-PAGE. By using EMA mAb and western technique, EMA was detected at 130 kDa but not in sera of healthy individuals samples. (Imam and Tökés, 1981) purified EMA under reduced condition using SDS-PAGE and identified single band at 70 kDa. (Imam et al., 1982) isolated two types of EMA using gel filtration and lectin affinity chromatography at 155 kDa and 39 kDa, respectively. (Greenwalt and Mather, 1985) isolated EMA from bovine milk at 76 kDa. (Hendrick et al., 1988) purified EMA at 35-1500 kDa. (Attallah et al., 2011) detected EMA at 130 kDa, as a single immunoreactive band in patients with liver fibrosis stages. (Bojić-Trbojević et al., 2014) showed similar bands ranging from 30-200 kDa using western blot analysis with anti-bovine submaxilllarymucin (BSM) and the well-known mucin antibodies which are human milk fat globulin and tumor associated glycoprotein. The variation of EMA molecular
weights (350-400 kDa) due to the difference of sources and the difference of antibodies used to detect EMA (Bhavanandan et al., 1998).

(Chaiteerakij et al., 2015) reported that advanced chromatography and mass spectrometry technologies are enabling the detection of small-molecule metabolites produced by dysregulated metabolic pathways during hepatocarcinogenesis. Canavaninosuccinate, an organic acid metabolite produced in the liver, is a marker for early HCC diagnosis. The serum canavaninosuccinate out performed AFP has 79% sensitivity and 100% specificity. These values are lower than the diagnostic performances of EMA. (Gomaa et al., 2009) reported that ultrasound scanning had low diagnostic performances in diagnosis of HCC patients. US have a sensitivity of 65%-80% and has a specificity of > 90%. This means that the sensitivity and the specificity of EMA in this study is higher than US. Several authors used epithelial membrane antigen as a tumor marker for hepatic tumors by using immunostaining technique. (Sloane and Ormerod, 1981) identified epithelial membrane antigen in human tissues by immunostaining techniques which used anti- serum raised against human milk fat globule membranes to stain a wide variety of human tissues by the indirect immunoperoxidase method for detection of epithelial membrane antigen. Epithelial membrane antigen can be used as marker of epithelial differentiation.

(Ormerod et al., 1983) identified EMA in cancer cells in liver and bone marrow. While (Bonetti et al., 1983) used immunoperoxidase technique for detection of EMA in 16 HCC patients. They found 14 out of 16 HCC patients were completely negative. Also (Sacho et al., 1991) emphasized that EMA staining is useful for more accurate classification of hepatic tumors. (Christensen et al., 1989) reported that the EMA stained 25 of 62 of HCC patients (40.3%). MUC1 Immunostaining were strong positive at 70.8%, 6.2% weak positive in HCC tissues, 25 % weak positive in liver cirrhosis tissues while all normal liver tissues were negative (Yuan et al., 2005).

Conclusion
In conclusion, epithelial membrane antigen was identified in serum of HCC by using EMA mAb and western blot technique at 130 kDa. Since, the EMA was detected by ELISA with a sensitivity of 81% and a specificity of 100%, EMA can be used in HCC diagnosis.

Disclosure
The authors declare that they have no competing interests. The study was approved by the Institutional Review Board.

References


