Evaluation of Serum Osteopontin Level as a Marker for Hepatocellular Carcinoma in Egyptian Patients

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Abstract: Background: Outcome of hepatocellular carcinoma (HCC) depends mainly on its early diagnosis. Biomarkers of HCC are helpful in screening, diagnosis and follow up of cases. The performance of traditional biomarkers is not satisfactory. Osteopontin (OPN) is a glycoprotein secreted by osteoblasts, osteoclasts, macrophages and T cells, and is over-expressed in a variety of tumors, including carcinomas of liver, stomach, breast, lung, colon, and prostate. The aim of this study was to identify a biomarker that could improve alpha-fetoprotein (AFP) performance in HCC surveillance among Egyptian patients with cirrhosis. **Methods:** The study population included 80 subjects divided into three groups. Group I: included 30 patients with HCC (proved by combined spiral computed tomography and ultrasonography), Group II: included 30 patients with liver cirrhosis (proved by clinical, laboratory and ultrasonographic findings), Group III: included 20 healthy subjects serving as controls. The serum level of OPN and alpha-fetoprotein (AFP) for all participants were assessed. **Results:** OPN plasma levels were significantly elevated in HCC patients, compared to cirrhosis, or healthy controls. OPN had higher sensitivity (93.3%) than AFP (42.6%) for selective detection of the HCC group over the non-HCC groups. OPN alone or in combination with AFP had significantly better area under the receiver operating characteristic curve, compared to AFP. OPN also had a sensitivity (74.33%; 95% CI: 60.29-88.81) in AFP-negative HCC. The sensitivity reached (100%; 95% CI: 71.51%-100.00%) when both serum levels were elevated. **Conclusion:** OPN was more sensitive than AFP for the diagnosis of HCC. These data propose elevated serum OPN levels as a potential biomarker for HCC in Egyptian patients.

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1. Introduction:

Hepatocellular carcinoma (HCC) is an increasingly prevalent clinical problem worldwide and is the third most common cause of cancer-related death ***(Venook et al., 2010)***. Cirrhosis of any etiology is the most common risk factor for HCC development. Over 90% of HCCs develop on a cirrhotic liver resulting from either chronic hepatitis B virus (HBV) or hepatitis C virus (HCV) infections, alcohol abuse, or accumulation of fat referred as nonalcoholic steatohepatitis ***(Sanyal et al., 2010)*** and ***(Bugianesi, 2007).***

Patients at risk for developing HCC should be entered into surveillance programs. Alpha-fetoprotein (AFP) is widely used as a surveillance and detection test for HCC among patients with cirrhosis, despite its limited performance, particularly in early-stage HCC ***(Daniele et al., 2004).***

Screening strategies including alpha-fetoprotein (AFP) and ultrasound every 6 months in patients with liver cirrhosis have been recommended to detect HCC at earlier stages leading to effective treatment strategies. AFP, however, is a marker with poor sensitivity and specificity and ultrasound is highly dependent on the operator's experience ***(Spangenberg et al., 2006).***

Apart from AFP, other markers (e.g., lectin bound AFP [AFP-L3], des-gamma carboxyprothrombin [DCP], and glypican-3) have been proposed for HCC detection ***(Stefaniuk et al., 2010)*** and ***(Villanueva et al., 2010).***

However, recent studies showed that neither DCP nor AFP-L3 presented better performance characteristics than AFP for the diagnosis of HCC ***(Marrero et al., 2009)*** and that neither DCP nor AFP is optimal to complement ultrasound in the detection of early HCC ***(Lok et al., 2010).***

Development of new biomarkers for the early detection of HCC thus remains an important target before a breakthrough appears on HCC surveillance and early intervention. So, the use of cancer biomarkers to anticipate the outlines of disease has been an emerging issue, especially as cancer treatment has made such positive steps in the last few years ***(Rodrigues et al., 2007).***

Osteopontin (OPN) is a phosphorylated glycoprotein secreted by activated macrophages, leukocytes, and activated T lymphocytes. Over-expression of OPN has been found in a variety of cancers, including carcinomas of stomach ***(Ue et al., 1998)***, breast ***(Rudland et al., 2002)***, prostate ***(Forootan et al., 2006)***, lung ***(Chambers et al., 1996)***, colon ***(Agrawal et al., 2002)***, and liver ***(Pan et al., 2003).***

OPN over-expression tended to be associated with the presence of tumor vascular invasion and advanced tumor grade, thus, indicating poor prognosis for patients with HCC, it may also have predictive potential for HCC invasion and metastasis ***(Zhang et al., 2012)***. Also it was found that interference of OPN expression inhibits the invasion and metastasis of human HCC ***(Lin et al., 2009).***

The aim of this study was to identify the clinical significance of OPN as a biomarker that could improve AFP performance in HCC surveillance among Egyptian patients with cirrhosis.

2. Patients and methods:

This study was conducted on 60 patients (after approval of the ethical committee); they were selected from the Tropical Medicine Department, Al-Azhar University and 20 healthy subjects as control group. Patients were divided as follow: Group I: 30 patients with hepatocellular carcinomas (proved by triphasic CT and abdominal ultrasonography); Group II: 30 patients with cirrhosis (diagnosed by clinical, laboratory and ultrasonographic examination). Group III: 20 apparently healthy subjects, age and sex matched, having no acute or chronic illness and taking no medications, were included as control group.

Exclusion criteria were: (1) Patients with any other tumor than HCC; (2) Patients with metastases of HCC; (3) Patients with hepatic focal lesions other than HCC; (4) Patients with bony lesions or inflammatory diseases and (5) Patients with previous HCC treatment.

All patients and controls (after informed consent) were subjected to: (A) History taking, (B) Clinical examination, (C) Liver and other biochemical profiles including: AST, ALT, serum albumin, total bilirubin, prothrombin time, INR, serum creatinine, and CBC. All were assayed using Hitachi auto analyzer and the kits were supplied from Roche Diagnostic, Germany. (C) Abdominal ultrasound with special emphasis on the liver, focal hepatic lesions, spleen, evidence of portal hypertension, abdominal lymph node enlargement and presence or absence of ascites. (D) Viral markers: Sera were tested for HBsAg and anti-HCV Ab by ELISA, using third generation kits (DiaSorin, Italy). (E) Serum Alpha-fetoprotein assay. (F) Serum Osteopontin level measuring. (G) Computed tomography (CT) abdomen and chest with bone scan (done for patients only) to exclude metastases of HCC.

Serum Osteopontin assay

Serum Osteopontin (OPN) was measured by enzyme linked immunosorbent assay (ELISA) using recombinant human OPN ELISA kit. Glory Science Co., Ltd. 2400 Veterans Blvd. Suite 16-101, Del Rio, TX 78840, USA.

Serum alpha-fetoprotein assay

Serum Alpha-fetoprotein (AFP) was measured by human AFP EIA kit lot. REF 600-10 manufactured by CanAg Diagnostics AB, Majnabble Terminal SE-414 55 Gothenburg, and Sweden.

Statistical methods

The SPSS 10.0 for windows was used for data management and analysis. Quantitative data were presented as mean ± SD. For comparison of the two groups' means, the Student's t-test was used, while for the comparison of the three groups' means, one way analysis of variance (ANOVA) was used followed by Post Hoc test. Non parametric quantitative data were expressed as median (range), Mann-Whitney tests were used for comparison of means. Qualitative data was expressed as frequency and percentage. Association between qualitative data was done using Chi-square test. To study the relationship between two variables Spearman's correlation coefficient was calculated. To summarize test performance on the whole range of thresholds, receiver operating characteristic (ROC) curves were plotted for OPN biomarker test. Area under the ROC curve (AUC) and its 95% confidence interval (CI) was calculated. Differences of sensitivities and specificities between OPN and AFP for differentiating HCC and their 95% CIs were calculated. All tests were two tailed and considered statistically significant at (p value < 0.05).

3. Results:

Group I included 21 males and 9 females with mean age 60.9 ± 9 years, group II included 13 males and 17 females with mean age 56.5 ± 9.3 years while group III included 13 males and 7 females with mean age 30.4±9.2 years (Table 1).

Table (1): Some demographic features of the studied groups.

	Group I (HCC) N = 30	Group II (cirrhosis) N = 30	Control group N= 20
Age Mean ± SD	60.9 ± 9	56.5 ± 9.3	30.4±9.2
Sex Male Female	21 (70%) 9 (30%)	13 (43.33%) 17 (56.67%)	13 (65%) 7 (35%)

Abdominal enlargement, abdominal pain and weight loss were the most common clinical presentations in group I (66.67%, 50% and 43.33% respectively), while abdominal enlargement, abdominal pain and jaundice were the most common clinical presentations in group II (70%, 40% and 40% respectively) (Table 2).

Table (2): Clinical presentations of the studied patients.

Clinical presentations	Group I (HCC) N = 30	Group II (cirrhosis) N = 30
Abdominal pain	15 (50%)	12 (40%)
Jaundice	10 (33.33%)	12 (40%)
Disturbed consciousness	6 (20%)	11 (36.67%)
Abdominal enlargement	20 (66.67%)	21 (70%)
Fever	5 (16.67%)	10 (33.33%)
Weight loss	13 (43.33%)	10 (33.33%)

Group I included 27 patients with chronic HCV infection (90%), 2 patients with chronic HBV infection (6.67%) and only 1 patient with co-infection of HCV & HBV (3.33%), while group II included 29 patients with chronic HCV infection (96.67%) and 1 patient with chronic HBV infection (3.33%) (Table 3).

Table (3): Viral markers of the studied patients.

Viral markers	Group I (HCC) N = 30	Group II (cirrhosis) N = 30
Anti-HCV Ab	27 (90%)	29 (96.67%)
HBs Ag	2 (6.67%)	1 (3.33%)
HCV Ab + HBs Ag	1 (3.33%)	0 (0%)

Group I included 4 patients with Child A cirrhosis (13.33%), 6 patients with Child B cirrhosis (20%) and 20 patients with Child C cirrhosis (66.67%), while group II included 3 patients with Child A cirrhosis (10%), 16 patients with Child B cirrhosis (53.33%) and 11 patients with Child C cirrhosis (36.67%) (Table 4).

Table (4): Severity of liver cirrhosis assessed by child-Pugh classification among the studied patients.

Child-Pugh class	Group I (HCC) N = 30	Group II (cirrhosis) N = 30	p-value
Child A (1-6)	4 (13.33%)	3 (10%)	0.72
Child B (7-9)	6 (20%)	16 (53.33%)	0.52
Child C (10-15)	20 (66.67%)	11 (36.67%)	0.69

By ultrasound, the frequencies of hepatomegaly, splenomegaly, ascites and portal vein thrombosis were 13.33%, 73.33%, 66.67% and 16.67% in group I, and 3.33%, 80%, 50% and 6.67% in group II respectively (Table 5).

Table (5): Ultrasonographic features of the studied patients.

Ultrasonographic feature	Group I (HCC) N = 30	Group II (cirrhosis) N = 30
Hepatomegaly	4 (13.33%)	1(3.33%)
Splenomegaly	22 (73.33%)	24 (80%)
Ascites	20 (66.67%)	15 (50%)
Portal vein thrombosis	5 (16.67%)	2 (6.67%)

Table (6): Ultrasonographic features of the hepatic focal lesions in the HCC group.

Focal lesions	Group I (HCC) N = 30
Site	
Rt. Lobe	19 (63.33%)
Lt. lobe	2 (6.67%)
Both	9 (30%)
Size	
< 3 cm	2 (6.67%)
3-5 cm	10 (33.33%)
> 5 cm	18 (60%)
Number	
Single	17 (56.67%)
Two	5 (16.67%)
Multiple > 2	8 (26.67%)
Lymph node enlargement	5 (16.67%)

HCC were detected in the right lobe in 19 (63.33%) patients, in the left lobe in 2 (6.67%) patients and in both lobes in 9 (30%). Lesions were < 3 cm in 2 (6.67%) patients, 3-5 cm in 10 (33.33%) patients and > 5 cm in 18 (60%) patients. Seventeen (56.67%) patients had single lesion, five (16.67%) patients had two lesions and 8 (26.67%) had multiple lesions. Only 5 patients (16.67%) had abdominal lymph node enlargement (Table 6).

Statistical analysis of serum OPN and AFP levels in the 3 groups showed that there was significant difference between group I (HCC) and the other 2 groups (cirrhosis and control) (P < 0.005) (Table 7).

Statistical analysis of the OPN showed that the median serum OPN level was significantly higher in the HCC group than in the cirrhosis or control groups (P-value < 0.005) (Figure 1). Also, the median serum AFP level was significantly higher in the HCC group than in the cirrhosis or control groups (P-value < 0.005) (Figure 2).

Table (7): Comparison of Alpha Fetoprotein and Osteopontin in the 3 Groups

Serum marker	Group I (HCC) N = 30	Group II (cirrhosis) N = 30	Control group N= 20	p-value
AFP (ng/ml)	3003±256[1,2]	15.74±1.2	10.8±0.95	0.0047
Osteopontin (µg/L)	8.44±1.2[1,2]	3.17±0.8	1.37±0.2	0.000*

[1]: p-value < 0.005 relative to the control group.
[2]: p-value < 0.005 relative to the cirrhosis group.

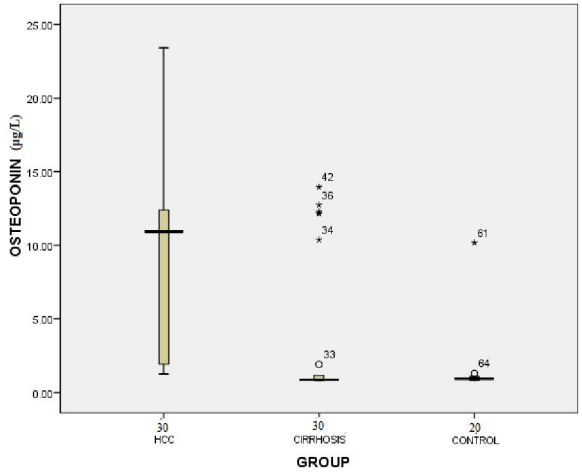


Figure 1. Serum Osteopontin levels in the three groups. Box plots represent median, quartiles and extremes, the asterisks represent outliers. HCC had higher serum OPN levels, compared with cirrhosis, and healthy control groups (P < 0.005).

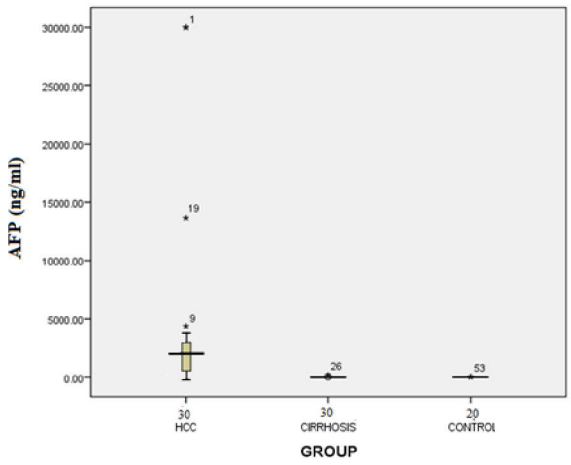


Figure 2. Serum AFP levels in different groups. Box plots represent median, quartiles and extremes, the asterisks represent outliers. HCC had higher serum AFP levels, compared with cirrhosis, and healthy control groups (P < 0.005).

The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of AFP for selective detection of the HCC group over the non-HCC groups (cirrhosis and healthy control groups) were 42.6%, 91.7%, 96.7% and 22% respectively; at a cut-off value 200 ng/ml (Table 8). While the sensitivity, specificity, PPV and NPV of OPN for selective detection of the HCC group over the non-HCC groups were 93.3%, 84%, 77.8% and

95.6% respectively, at a cut-off value 1.25 µg/L (Table 8, Figure 3).

Table (8): Diagnostic Sensitivity, Specificity, PPV and NPV of Plasma OPN Level of HCC Patients in Comparison With AFP for Selective Detection of HCC.

Test	Cut off	Sensitivity %	Specificity %	PPV %	NPV %
AFP (ng/ml)	200	42.6	91.7	96.7	22
Osteopontin (µg/L)	1.25	93.3	84	77.8	95.6

PPV: positive predictive value. NPV: negative predictive value.

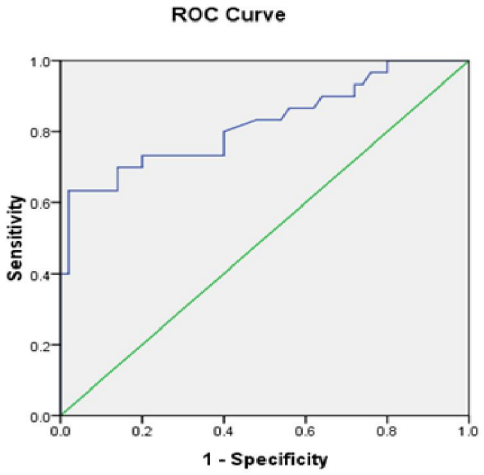


Figure 3. Receiver operating characteristics (ROC) curve analysis of serum OPN for discrimination between group 1 (HCC) and the other 2 groups.

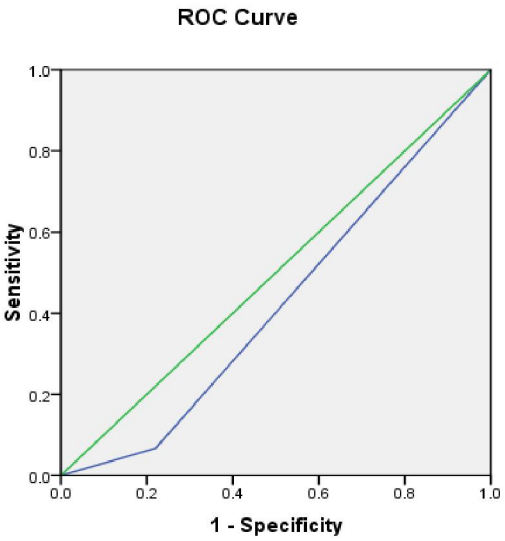
We evaluated the sensitivity, specificity, PPV and NPV with their 95% confidence intervals of AFP & OPN in discrimination between group 1 (HCC) and the other 2 groups (cirrhosis & control) when (serum AFP and OPN were normal) AFP- OPN-, (serum AFP was increased while serum OPN was normal) AFP+OPN-, (serum AFP was normal but serum OPN was increased) AFP-OPN+ and (when both serum levels were increased) AFP+OPN+ using AFP and OPN cut-off values. The sensitivity in AFP-OPN+ cases (74.33%; 95% CI: 60.29-88.81) was higher than in AFP+OPN- cases (50.00%; 95% CI: 1.26-98.74). The sensitivity reached (100%; 95% CI: 71.51%-100.00%) when both serum levels were elevated (Table 9).

Table (9): Sensitivity, Specificity, PPV and NPV of AFP- OPN-, AFP+OPN-, AFP-OPN+ and AFP+OPN+ Using AFP and OPN Cut-off Values.

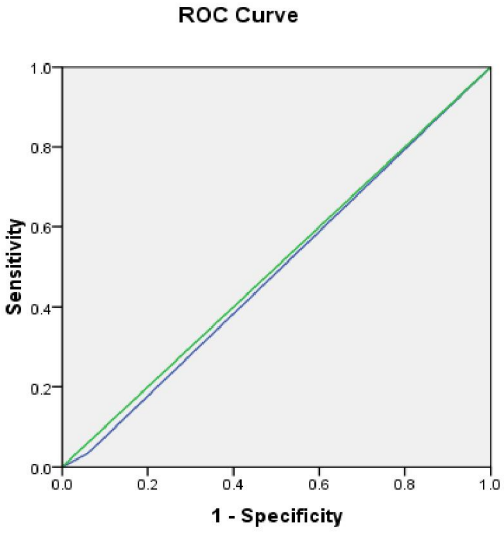
Test	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)
AFP < 200 ng/ml OPN < 1.25 µg/L	15.38% (1.92% - 45.45%)	58.21 % (45.52%-70.15%)	6.67% (0.82%-22.07%)	78.00 % (64.04%-88.47%)
AFP > 200 ng/ml OPN < 1.25 µg/L	50.00% (1.26% - 98.74%)	62.82 % (51.13% - 73.50%)	3.33% (0.08%-17.22%)	67.21 % (62.00%-91.69%)
AFP < 200 ng/ml OPN > 1.25 µg/L	74.33% (60.29% - 88.81%)	82.00 % (68.56% - 91.42%)	52.63% (28.86%-75.55%)	98.00 % (89.35%-99.95%)
AFP > 200 ng/ml OPN > 1.25 µg/L	100.00% (71.51%-100.00%)	72.46 % (60.38% - 82.54%)	36.67% (19.93%-56.14%)	100.00 % (92.89%-100.00)

AFP, alpha-fetoprotein; OPN, osteopontin; 95% CI, 95% confidence interval; PPV, positive predictive value; NPV, negative predictive value.

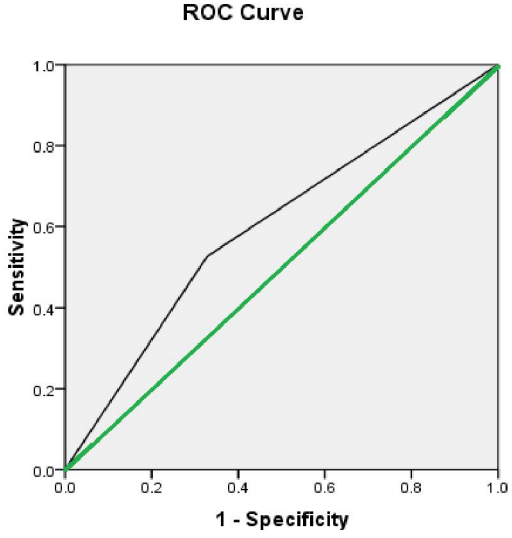
We evaluated the area under the curve (AUC) with their 95% confidence intervals of AFP and OPN in discrimination between group 1 (HCC) and the other 2 groups (cirrhosis & control) when (serum AFP and OPN were normal) AFP- OPN- (figure 4A), (serum AFP was increased while serum OPN was normal) AFP+OPN- (figure 4B), (serum AFP was normal but serum OPN was increased) AFP-OPN+ (figure 4C) and (when both serum levels were increased) AFP+OPN+ (figure 4D) using AFP and OPN cut-off values. The AUC for AFP-OPN+ cases (0.573, 95% CI: 0.456-0.716) (figure 4c) was higher than in AFP+OPN- cases (0.487, 95% CI: 0.356-0.617) (figure 4b). The combination of OPN and AFP further increased the AUC (0.683, 95% CI: 0.553-0.814) (figure 4c).



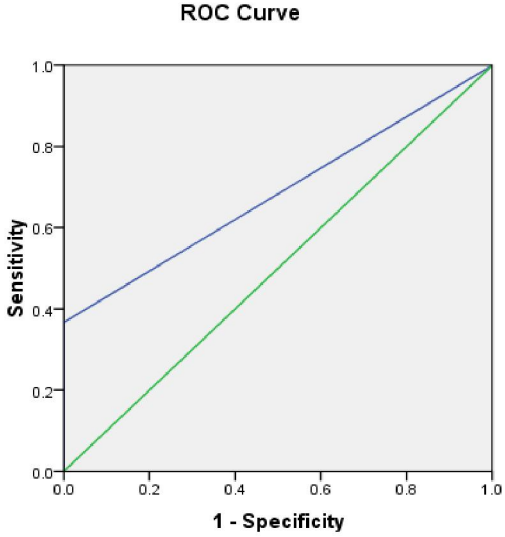
A
Osteopontin <1.25; AFP < 200
AUC: 0.423, 95%; CI: 0.297-0.550



B
Osteopontin <1.25; AFP > 200
AUC: 0.487, 95%; CI: 0.356-0.617



C
Osteopontin >1.25; AFP < 200
AUC: 0.573, 95%; CI: 0.456-0.716.



D
Osteopontin >1.25; AFP > 200
AUC: 0.683, 95%; CI: 0.553-0.814.

Figure 4. Receiver operating characteristics (ROC) curves analysis of sreum OPN and AFP for discrimination between group 1 (HCC) and the other 2 groups (cirrhosis & control). The AUC (area under the curve) is shown, together with the 95% CIs (confidence intervals) when AFP-OPN- (A), AFP+OPN- (B), AFP-OPN+ (C) and AFP+OPN+ (D) using AFP and OPN Cut-off Values.

Correlations between OPN and AFP and different parameters for all studied patients showed that serum AFP and OPN levels had no significant correlation to the Child-Pugh class, model for end-stage liver disease score (MELD score) or tumor size of the studied patients. We observed only an increase of serum OPN levels depending on the tumor multiplicity while AFP not. AFP only showed inverse significant correlation with the age of the patients. There was no any direct significant correlation between serum OPN and AFP levels in the studied patients (Table10).

Table (10): Correlations between OPN and AFP and different parameters for all studied patients (n=60).

Variable	AFP		OPN	
	r	p	r	p
Age (y)	- 0.39	0.03*	0.04	0.85
RBC's ($\times10^6$/mm^3)	- 0.03	0.90	- 0.32	0.08
WBC's ($\times10^3$/mm^3)	0.07	0.88	0.21	0.54
Hb (gm/dL)	0.02	0.92	- 0.21	0.27
PLT ($\times10^3$/mm^3)	0.22	0.72	0.18	0.89
T. Bilirubin (mg/dL)	0.05	0.77	- 0.03	0.73
AST (IU/L)	0.36	0.77	0.17	0.17
ALT (IU/L)	- 0.06	0.38	- 0.21	0.78
Albumin (gm/dL)	- 0.14	0.79	- 0.36	0.80
INR	0.17	0.05	- 0.17	0.51
ALP (IU/L)	- 0.17	0.46	- 0.16	0.05
Child-Pugh class	0.155	0.34	0.11	0.48
MELD score	0.18	0.246	0.158	0.33
Tumor number	0.154	0.344	0.33	0.036*
Tumor size (cm)	0.29	0.068	0.13	0.4
Osteopontin (µg/L)	0.03	0.88		

r: correlation coefficient. * : Significant correlation (p-value<0.05). p: p-value MELD: Model for end-stage liver disease

4. Discussion:

OPN is a secreted phosphoprotein that binds alphaV-integrins and cluster of differentiation (CD) 44 families of receptors ***(El-Tanani, 2008).*** Elevated expression of OPN has been associated with tumor invasion, progression, or metastasis in multiple cancers, ***(Anborgh et al., 2010)*** and OPN has been proposed as a promising target for cancer therapy ***(Johnston et al., 2008)***.

In HCC, elevated OPN is regarded as a potential prognostic biomarker, and overexpression of OPN is closely correlated with intrahepatic metastasis, early recurrence, and a worse prognosis ***(Pan et al., 2003).***

OPN expression is also critical for tumor growth of human HCC, and that down-regulation of OPN suppresses growth of HCC via induction of apoptosis ***(Zhao et al., 2008).***

Early detection of patients with HCC is an attractive goal because it gives better prognosis as HCC tends to grow slowly and stay confined to the liver. Early detection is possible with ultrasound scanning and AFP monitoring, although the use of AFP as a screening test is complicated by frequent false positive and false negative results ***(Gogel et al., 2000).***

In our study, most of the patients had anti-HCV sero-positivity due to its high prevalence rate which was reported by ***Nair et al. (2002).***

In our study, the age of HCC patients ranged from 39 to 70 years with a mean of 60.9 ± 9 years and this is probably attributed to the duration of the underlying liver disease, also ***Di Bsiceglie (2002)*** stated that HCC is reported to develop in the fifth decade. The same results were reported by ***Johnson (2000)***, who found that the average age of patients with HCC ranged from fifth to sixth decades of life.

The sex of patients, in our study, showed a male predominance in HCC patients with a male: female ratio 2.3: 1, this male predominance was also observed by ***Goldman and Ausiello (2004)***, who reported a male: female ratio 2:1 up to 4:1.

The clinical features of HCC are often similar to those caused by the underlying hepatic disease. It is very hard for physicians to distinguish signs and symptoms of HCC in contests characterized by an advanced liver disease ***(Trevisani et al., 1995).***

In our study, abdominal enlargement, abdominal pain and weight loss were the most common clinical presentations in HCC (66.67%, 50% and 43.33% respectively), while abdominal enlargement, abdominal pain and jaundice were the most common clinical presentations in cirrhotic patients (70%, 40% and 40% respectively).

Advanced liver cancer can be responsible for accelerated liver functions deterioration caused by the intrahepatic tumor growth. A large HCC can worsen the underlying hepatic disease, therefore in case of clinical worsening of a cirrhotic patient, onset of a HCC should be suspected ***(Lam et al., 2004).***

So in the present study, 20 patients (66.67%) in the HCC group were of Child C cirrhosis, 4 patients with Child A cirrhosis (13.33%) and 6 patients with Child B cirrhosis (20%), while group II included 3 patients with Child A cirrhosis (10%), 16 patients with Child B cirrhosis (53.33%) and 11 patients with Child C cirrhosis (36.67%).

Development of new biomarkers for the early detection of HCC thus remains an important target before a breakthrough appears on HCC surveillance and early intervention. So, the use of cancer biomarkers to anticipate the outlines of disease has been an emerging issue, especially as cancer treatment has made such positive steps in the last few years ***(Rodrigues et al., 2007).***

By ultrasound examination, most of the HCC lesions 19 (63.33%), in our study, were found in the right lobe of the liver. Similarly, ***Rosen and Nogarney (1997)*** documented that HCC occurs most frequently in the right lobe of the liver either as a solitary mass or as multiple nodules. This may be due to the large size of the right lobe of the liver which is 6 times the left lobe. Also, ***El-Kady et al. (2009)*** found in their study that 75-100% of focal lesions were found in the right lobe.

By studying AFP and OPN levels in different groups, we found significant elevation of serum OPN and AFP levels in HCC patients than cirrhotic patients and lower levels in normal control group was evident in our study. ***Kim et al. (2006)*** and ***Zhao et al. (2008)*** also found that median serum OPN level in HCC patients was higher than in patients with chronic liver disease. Also, ***Zhang et al. (2006)***, ***El-Din Bessa et al. (2010)*** and ***Abu El Makarem et al. (2011)*** found that the median serum OPN level was significantly higher in the HCC group than in the cirrhotic patients or in the normal control group.

Shang et al. (2012) evaluated the performance of OPN in discriminating HCC and cirrhosis patients. The AUC for OPN (0.76; 95% CI: 0.66-0.85) was higher than for AFP (0.71; 95% CI: 0.60-0.82). The combination of OPN and AFP further increased the AUC (0.82; 95% CI: 0.73-0.91).

Comparable results were obtained in this study. The sensitivity in AFP-OPN+ cases (74.33%; 95% CI: 60.29-88.81) was higher than in AFP+OPN- cases (50.00%; 95% CI: 1.26-98.74). The sensitivity reached (100%; 95% CI: 71.51%-100.00%) when both serum levels were elevated.

We evaluated the area under the curve (AUC) with their 95% confidence intervals (CI) of AFP and OPN in discrimination between group 1 (HCC) and the other 2 groups (cirrhosis & control). The AUC for AFP-OPN+ cases (0.573, 95% CI: 0.456-0.716) was higher than in AFP+OPN- cases (0.487, 95% CI: 0.356-0.617). The combination of OPN and AFP further increased the AUC (0.683, 95% CI: 0.553-0.814). Most of HCC patients with their AFP level less than 200 ng/mL showed high serum OPN level. Same results obtained by ***Shang et al. (2012)***.

Correlations between OPN and AFP and different parameters for all studied patients showed that serum AFP and OPN levels had no significant correlation to the Child-Pugh class, model for end-stage liver disease score (MELD score) or tumor size of the studied patients.

These results not coincide with the results obtained by ***Kim et al. (2006)*** who reported that within the HCC group, OPN levels correlated with progressive deterioration of underlying liver function in terms of Child-Pugh class and advancing degree of tumor stage.

Therefore, the significant increase of plasma OPN levels seemed to be relevant only to the process of carcinogenesis rather than cirrhosis or fibrosis. This is in agreement with the immunohistochemistry result, which shows that elevated plasma OPN is derived from malignant hepatocytes and tumor-infiltrating macrophages, not from the noncancerous hepatocytes or Kupffer cells ***(Shang et al., 2012)***.

We observed an increase of serum OPN levels depending on the tumor multiplicity while AFP not. AFP only showed inverse significant correlation with the age of the patients. This corresponded to the results of ***Zhang et al. (2006)***, as they found that the median plasma OPN level of patients with multiple tumor nodules (217.11 ng/mL) was higher than that of patients with a single tumor nodule (168.18 ng/mL).

There was no significant correlation between OPN level and tumor size in our study. The relation between OPN and tumor size was also studied by ***Zhang et al. (2006)*** and they found that tumors ≤ 5 cm showed median plasma OPN level 176.90 ng/mL, and tumors > 5 cm showed median plasma OPN level 172.92 ng/mL.

There was no any direct significant correlation between serum OPN and AFP levels in the studied patients and this was coincide with the results obtained by ***Kim et al. (2006)***.

Another important issue that limits the potential utility of plasma OPN levels as a specific biomarker for cancer is that OPN level is also increased in a range of inflammatory syndromes ***Matsuiet et al. (2004).*** Therefore, further careful evaluation with one standardized assay system will be needed to gain greater insight into the potential usefulness of OPN in Egyptian patients with HCC.

Conclusion:

OPN was more sensitive than AFP for the diagnosis of HCC. These data propose elevated serum OPN levels as a potential biomarker for HCC in Egyptian patients.

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