Osteopontin as hepatocellular carcinoma marker in HCV related liver cirrhosis

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Abstract: Background: α-Fetoprotein (AFP) is the biomarker most widely used to detect hepatocellular carcinoma (HCC), despite its suboptimal diagnostic accuracy. Osteopontin (OPN) is a secreted phosphoprotein which has been linked to tumor progression and metastasis in a variety of cancers including hepatocellular carcinoma (HCC). **Aim of the work:** This study was conducted to evaluate the clinical utility of using plasma OPN as diagnostic markers for HCC in comparison with AFP. **Patients and methods:** 150 subjects were enrolled. They were divided into three groups: (group I) include 50 HCV cirrhotic patients with HCC, (group II) include 50 HCV cirrhotic patients without HCC and (Group III) include 50 healthy subjects as control group. Patients who underwent previous treatment for HCC and patients with other malignancies either in the past or recently diagnosed and patients with cirrhosis of any etiology other than HCV were excluded. All subjects underwent history taking, clinical examination, and laboratory investigations including: CBC, BUN, creatinine, sodium, potassium, ALT, AST, albumin, total bilirubin, INR, plasma osteopontin (OPN) and serum alpha fetoprotein (AFP). Cirrhotic patients included underwent abdominal ultrasound and triphasic CT abdomen. **Results:** HCC patients had the highest levels of AFP and OPN ($p< 0.001$). OPN area under receiver operating characteristic curve (AUROC) for HCC diagnosis was 0.991 (CI 95%: 0.948 to 1.000) with p <0.0001. OPN > 178 ng/ml had sensitivity and specificity of 98% and 96% respectively for HCC diagnosis.AFP AUROC was 0.889 (CI 95% 0.810 to 0.943) with <0.0001. AFP > 185 ng/ml had sensitivity of 86% and specificity of 94% in diagnosing HCC.OPN had better diagnostic performance than AFP (OPN AUROC= 0.991 vs AFP AUROC=0.889) (p=0.01). **Conclusion:** OPN is a useful biomarker for HCC diagnosis with better performance than AFP.

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1. Introduction:

Osteopontin (OPN) is a glycoprotein that was first identifiedin 1986 in osteoblasts. The prefix 'osteo' indicates that the protein is expressed in bone, the suffix 'pontin' is derived from 'pons' the Latin word for bridge that signifies osteopontin's role as a linking protein**(1)**.

Osteopontin is an extracellular structural protein composed of ~ 300 amino acids residues and has ~ 30carbohydrate residues attached including ten sialic acid residues. Although highly expressed in bone, OPN is also expressed by various cell types including macrophages, endothelial cells, smooth muscle cells and epithelial cells (2).The putative functions of Osteopontin are bone mineralization, regulation of immune cell function, inhibition of calcification, control of tumor cell phenotype and cell activation (3).

Osteopontin expression and secretion by tumor cells has been shown to enhance the invasive potential of cancer cells and plays an important role in cancer progression (4). However, the interaction between tumor-secreted OPN and macrophages that facilitates tumor progression and metastasis still remains unclear in clinical practice (5). In studies on humans, OPN expression has been found in carcinomas of colon, pancreas, multiple myeloma, and other tumor types (6).

Hepatocellular carcinoma (HCC) is an increasingly prevalent clinical problem worldwide and is the third most common cause of cancer-related death (7). 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 non-alcoholic steatohepatitis (NASH) (8).

Hepatocellular carcinoma (HCC) is an aggressive tumor that is different from other cancers in terms of its frequent recurrence or metastasis after curative therapy (9). In most cases, HCC is diagnosed at an advanced stage, and often arises in a background of chronic liver disease and cirrhosis. Therefore, the prognosis of patients with HCC is generally poor, with a 5- year survival rate of less than 5% (10).Because the poor outcomes of HCC patients are often related to late detection, recent practice guidelines recommend continued surveillance for patients at high risk (11).

Screening procedures for HCC include serological and radiological tests, and among the serological tests, α-fetoprotein (AFP) and prothrombin induced by vitamin K absence II (PIVKA II) are widely used as biomarkers for HCC (12)(13) (14). However, ~30% of HCC patients are negative for AFP and PIVKA II, and screening for these biomarkers may not be satisfactory due to low sensitivity and specificity (15). Therefore, tumor markers with better diagnostic accuracy are urgently needed For HCC.

HCCs were shown to consistently express OPN at higher levels than normal tissues (16).There have been studies reporting the use of plasma OPN as a marker for HCC (17-19), but its diagnostic value remains to be a debate when compared with AFP. Therefore our study aimed at evaluating the diagnostic value of plasma OPN compared with AFP for the diagnosis of HCCin HCV related liver cirrhosis.

2. Patients and Methods

This study had been carried out on 150 subjects.Subjects were divided into three groups: (group I) include 50 HCV cirrhotic patients with HCC, (group II) include 50 HCV cirrhotic patients without HCCand (Group III) include 50 healthy subjects as control group.

HCV related liver cirrhosis and HCC diagnosis were confirmed based on clinical, laboratory and radiological data. Written informed consent was obtained from each subject before enrollment in the study and the study was approved from the Local Ethical Committee of Ain Shams University.

Patients who underwent previous treatment for HCC and patients with other malignancies either in the past or recently diagnosed and patients with cirrhosis of any etiology other than HCV were excluded.

All subjects underwent complete history taking, clinical examination, and laboratory investigations including: CBC, BUN, creatinine, sodium, potassium, ALT, AST, albumin, total bilirubin, INR, plasmaosteopontin (OPN) and serum alpha fetoprotein (AFP). Plasma osteopontin (OPN) was measured by Enzyme Linked Immunosorbent assay (ELISA) using recombinant human OPN ELISA kit (R&D® Systems, Inc. Minneapolis, United States of America).Serum alpha fetoprotein (AFP) was measured by human AFP EIA kit (Canag® Diagnostics AB, Gothenburg, Sweden).

Cirrhotic patients included in our study also underwent abdominal ultrasound and triphasic CT abdomen.

Statistical Methods:

Statistical analysis was performed using SPSS v.18.0 (IBM Corp., Armonk, NY, USA). Quantitative variables were described as mean and standard deviation (SD) while qualitative variables were described as frequency and percentage. For comparison of 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. Receiver operating characteristic (ROC) curves were constructed and the corresponding areas under the ROC curve (AUROC) were computed for each of the noninvasive indices. The sensitivity and specificity were calculated using the ROC curves. Cutoff points were determined as the value corresponding with maximum sensitivity and specificity. Significance level was expressed as $P<0.05$.

3. Results

A total of 150 subjects were enrolled in the study. Study subjects fell into 3 equal groups (n=50): group (I) cirrhotic patients with HCC, group (II) cirrhotic patients without HCC and group (III) healthy subjects as control group. Table 1 shows descriptive statistics of the three groups.

Table 1- Descriptive statistics of the study patients' groups

Parameters	I	II	III
Age	51.7±4.15	51.06±6.01	42.36±13.42
Sex Male Female	 42(84%) 8(16%)	 30(60%) 20(40%)	 24(48%) 26(52%)
Ascites	35 (70%)	25 (50%)	None
AST(IU/L)	74.8 ± 19.9	62.2 ± 20.6	18.8 ± 5.6
ALT(IU/L)	53.2 ± 18.2	37.8 ± 13.7	27.9 ± 8.01
Albumin (gm/dl)	2.5 ± 0.6	2.5 ± 0.54	4.38 ± 0.56
Total bilirubin (mg/dl)	5.1 ± 3.6	3.5 ± 3.2	0.73 ± 0.2
BUN (mg/dl)	16.5 ± 5.8	19.9 ± 10.1	14.2 ± 4.2
Creatinine(mg/dl)	0.87 ± 0.31	0.9 ± 0.26	0.82 ± 0.21
INR	1.7 ± 0.5	2.1 ± 0.7	0.85 ± 0.16
TLC (10^3/mm^3)	5.5 ± 2.1	5 ± 2	7.5 ± 1.3
Hb (gm/dl)	9.9 ± 1.8	10 ± 1.5	13 ± 0.9
Platelets (10^3/mm^3)	67 ± 35	61 ± 31	285 ± 96

39 (78%) patients had single HCC and 28 (56%) patients had their largest HCC lesion measuring ≥ 3 cm(Table 2).

Comparisonof AFP and OPN levels in the three groups using ANOVA test revealed significant difference between the three groups (p< 0.001) with HCC patients had the highest levels as regards AFP and OPN (Tables 2, 3).

Table 2- Tumor characteristics by triphasic CT abdomen in group I patients

Variable	Description*(n,%)*	
Tumor size	<3 cm	22 (44%)
	≥ 3 cm	28 (56%)
Multiplicity	Single	39 (78%)
	Multiple	11 (22%)

Table 3-Comparison between study groups as regard AFP and OPN

Parameters	I	II	III	*P* value
AFP (ng/ml)	910.9±588.48	70.72±62.16	4.64±2.69	< 0.001
OP (ng/ml)	363.93±129.14	123±43.03	49.32±20.88	< 0.001

Assessing the diagnostic value of osteopontin in HCC, area under receiver operating characteristic curve (AUROC) was 0.991 (CI 95%: 0.948 to 1.000) with p <0.0001 which is highly significant (Figure 1). OPN > 178ng/ml had the best combined sensitivity and specificity (98% and 96% respectively) (Table 4).

AFP AUROC for HCC diagnosis was 0.889 (CI 95% 0.810 to 0.943) with <0.0001 (Figure 2).at cutoff value of AFP > 185 ng/ml, AFP had sensitivity of 86% and specificity of 94% in diagnosing HCC (Table 4).

Comparison between AFP and OPN in HCC diagnosis showed a significant difference between both markers (P = 0.01) with better performance of OPN (OPN AUROC= 0.991 vs AFP AUROC=0.889) (Table 4, Figure 3).

Table 4-OPN and AFP in HCC diagnosis

Parameter	OPN	AFP
AUROC	0.991	0.889
95% CI for AUROC	0.948 to 1.000	0.810 to 0.943
Cutoff value	> 178	> 185
Sensitivity	98%	86%
Specificity	96%	94%
Comparison between ROC curves	Difference between areas =0.103; Significance level P = 0.01	

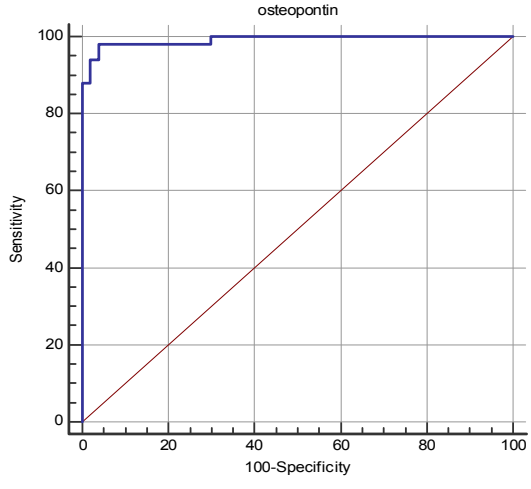

Figure 1-ROC curve for OPN in HCC diagnosis

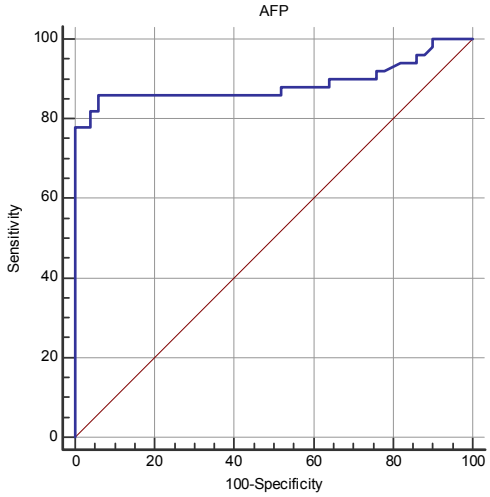

Figure 2-ROC curve for AFP in HCC diagnosis

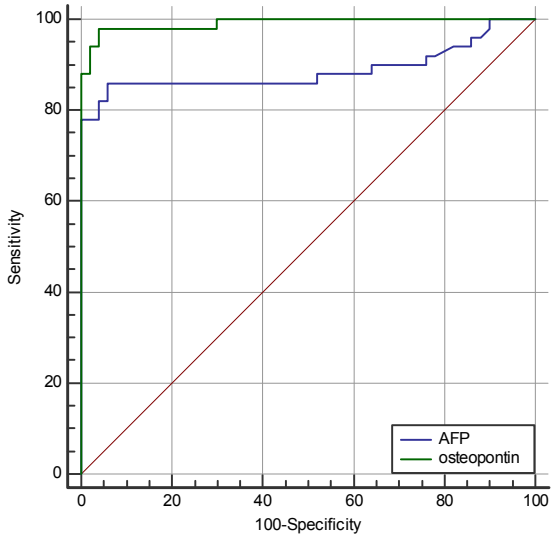

Figure 3- Comparison of ROC curves of AFP and OPN in HCC diagnosis.

4. Discussion:

Osteopontin (OPN) is a multifunctional cytokine that impacts cell proliferation, survival, drug resistance, invasion, and stem like behavior. Its aberrant expression and/or splicing is functionally responsible for undesirable alterations in disease pathologies, specifically cancer. OPN has been implicated as a prognostic and diagnostic marker for several cancer types (20). The current study aimed at assessing OPN value in HCC diagnosis in HCV patients compared with AFP.

The three groups included in our study differed significantly ($p < 0.001$) as regard AFP and OPN levels with group I (cirrhotic patients with HCC)had significantly higher levels than group II and III (cirrhotic patients without HCC and healthy controls respectively). Also group 2 patients had significantly higher ($p < 0.001$) OPN and AFP levels than group 3 healthy controls.

This agrees with the study by ***El-Din Bessa et al.*** with plasma levels of OPN and AFP in HCC cirrhotic patients (n= 30) being significantly higher than in cirrhotic patients without HCC (n=30) and healthy controls (n=20) (p <0.001) (21). This also agrees with ***Kim et al.*** who determined plasma levels of OPN, alpha-fetoprotein (AFP), in a group of 62 HCC patients, in 60 patients with chronic liver diseases, and in 60 healthy control individuals showing thatplasma OPN levels in the HCC patients were significantly higher ($p < 0.001$) than those patients with chronic liver diseases or of a healthy control group (22). Similar results were also reported by ***Nabih et al.***(17). ***Salem et al.*** found that osteopontin levels were significantly elevated in 30 patients with HCC and in thirty HCV patients in comparison to control group (10 healthy subjects) (p: 0.005) (23).Similarly, ***Abu El Makaremet al.*** reported that the median plasma OPN level was significantly higher in the HCC group than in the cirrhotic patients group or in the normal control group ($p < 0.001$) (24).

Assessing the diagnostic value of OPN in HCC, we found that OPN AUROC for HCC diagnosis was 0.991 (95% CI: 0.948 to 1.000) and it differed significantly (p = 0.01) from AFP AUROC (0.889, 95% CI: 0.810 to 0.943). At a cutoff value of OPN > 178 ng/ml, the test had sensitivity of 98% and specificity of 96% while AFP at a cutoff value of >185 ng/ml had sensitivity and specificity of 86% and 94% respectively in HCC diagnosis.

Abu El Makarem et al.(24) reported AUROC for OPN was (0.998; 95% Confidence Interval (CI):0.952-1) which was significantly (p= 0.0001) higher than that yielded by AFP (0.91; with 95% CI: 0.826-0.961). The sensitivity, specificity, of plasma OPN were 97.67% and 100%, at a cut-off value of 300 ng/ml. For AFP at a cut-off value > 43 ng/mL; the values of sensitivity, specificity, were 74.4%, 100% respectively.

A meta-analysis of 8 studies (4 for prognosis and 4 for diagnosis, 1399 patients) was done by ***Cheng et al.*** The summary estimates for plasma OPN and AFP in diagnosing HCC in the studies included were as follows for OPN sensitivity, 88% (95% CI: 84%-91%), specificity, 87% (95% CI, 83%-90%) and AUROC 0.91 (95% CI, 0.85-0.97). while for AFP, sensitivity was68% (95% CI: 63%-73%),specificity 97% (95% CI, 94%-99%); and AUROC 0.68 (95% CI, 0.45-1.03).The performance of OPN and AFP was better in our study than those reported in **Cheng *et al.*** meta-analysis (25).

Although reporting better diagnostic accuracy of OPN over AFP in HCC diagnosis which agrees with our study results, many studies reported lower performance of OPN and AFP than we found. ***El-Din Bessa et al.*** reported the sensitivity and specificity of OPN for HCC diagnosis were 88.3% and 85.6%, respectively, at a cut-off value of 9.3 ng/mL with OPN having a greater area under curve value (0.918) than AFP (0.712) (21). Also ***Kim et al.*** found that the diagnostic sensitivity and specificity of OPN for HCC was 87% and 82%, respectively (cut-off value: 617.6 ng/mL) with OPN had a greater area under curve value (0.898) than AFP (0.745)(22).The meta analysis of seven studies by ***Wan et al.*** estimated OPN and AFP sensitivity and specificity as follows: sensitivity, 0.86 (0.79-0.91) vs 0.66 (0.53-0.76), specificity, 0.86 (0.69-0.94) vs 0.95 (0.87-0.98), and the area under the curve (AUC), 0.92 vs 0.87 (18).Also, ***Nabih et al.*** Receiver operator characteristic (ROC) curves showed that the area under the curve (AUROC) for OPN and AFP was 0.824 and 0.730, respectively (17).

The performance of OPN in diagnosis of HCC in our study is much better than that reported by ***Lee et al.*** with area under the ROC curve (AUROC) value for OPN 0.51 which is much lower than our value (0.991) (19). Similarly, ***Salem et al.*** reported much lower sensitivity and specificity of OPN for detection of HCC (73% and 54%, respectively) at a cut-off value of 128.5 ng/mL (23).

Conclusion:

Our findings suggest that osteopontin is a valuable marker for HCC diagnosis with better performance than AFP. Validation of osteopontin value either alone or in combination with AFP in HCC diagnosis over larger population of HCC patients is needed. Also assessment of the response of osteopontin levels to intervention and its relation to prognosis and recurrence of HCC is recommended.

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