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Research Article

Diagnostic Value of Serum Syndecan-1 in Hepatocellular Carcinoma Associated with Hepatitis C virus in Egyptian Patients

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ABSTRACT

Hepatocellular carcinoma (HCC) can benefit from tumor biomarkers' diagnostic, therapeutic, and prognostic capabilities. There is a need for the use of accurate, rapid and inexpensive tumor marker with ultrasonography and computer tomography in appropriate diagnosis of HCC.

The present study was designed to investigate the potential role of syndecan-1 as a diagnostic and prognostic, non-invasive biomarker for HCC. Also, to assess its accuracy, sensitivity and specificity in comparison with the usual recommended biomarker α-fetoprotein (AFP).

This study is a cross sectional case–control study. The study included 45 patients with HCC associated with chronic HCV infection. In addition, 20 healthy subjects were included as a control group. AFP and syndecan-1were determined by enzyme immunoassay.

Syndecan-1 serum level showed significantly marked elevation in HCC patients compared to control subjects ($P<0.001$). Analytical correlation study revealed highly significant positive correlation between syndecan-1 serum level with older patient over 55 years old , larger tumor size more than 2.5 cm and the presence of ascites ($P<0.001$). Moreover, there was statistically significant increase in syndecan-1 levels in advanced Child-Pugh classifications ($P<0.001$). For determination the best diagnostic performance level of syndecan-1 represented by cut off value, receiver operative curve was drawn (ROC). Cut off point level were 4241 pg/ml with sensitivity 99.8% and 85% specificity.

We conclude that syndecan-1 is an accurate biomarker in the diagnosis and prognosis of HCC, since its level is elevated in HCC patients and correlated with tumor size, patients over 55 years old, larger tumor size more than 2.5 cm, the presence of ascites and different stages of HCC. Further studies on large groups of patients are required to validate these results.

Keywords: HCC, Syndecan-1, α-fetoprotein

INTRODUCTION:

Hepatocellular carcinoma (HCC) is the most common primary malignancy of the liver, the third most cancer type and is considered the second most frequent cancer in men in Egypt. Hepatitis C virus (HCV) is a common infection in Egypt and considered the main cause of HCC. There were reports identified the increase of frequency of HCC from 4.0% in 1993 to 7.3% in 2003 in Egypt (1). The success of management of HCC depends mainly upon early detection. There is effective treatment for early stage HCC including surgical resection and radio frequency ablation. Yet advanced stage of HCC has limited therapeutic options with poor prognosis (2). Unfortunately more than two-thirds of patients are discovered at advanced stages (3). The principle detection methods depend mainly on ultrasonography and computer tomography. The use of tumor biomarkers in blood may provide an easy and rapid method for screening of susceptible patients (4).

The approved screening methods for early detection of HCC in patients with HCV depend mainly on combination of ultrasound scanning and serum α-fetoprotein (AFP) assay every 6 to 12 months (5). Ultrasound surveillance even performed at every three month intervals cannot improve detection of small HCC because of limitations in recall procedures (6, 7). However, this method has limited sensitivity. Though

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detection of AFP has high sensitivity for early detection of HCC (8), it appears to be less sensitive in early detection of HCV associated HCC especially in African ethnicity (9).

Thus a need arises for the use of new biomarkers that can aid in early diagnosis and prognosis of HCC associated with HCV. Several molecules have been investigated to be used as tumor markers like growth factors and their receptors, tumor-specific growth factor (TSGF), epidermal growth factor receptor family, hepatocyte growth factor/scatter factor (10) and Syndecan-1.

The syndecan family consists of four transmembrane heparin sulfate proteoglycans (HSPGs) mainly present on the cell surface (11, 12). The structures of these different syndecans show high homology in vertebrates and invertebrates (6, 7). All four syndecans are built up of a core protein decorated with varying number of glycosaminoglycan (GAG) side chains. Syndecans exert their functions mainly through these GAG chains, but the different domains of the core protein have distinct roles as well (13, 14). Syndecan-1 and syndecan-3 carry both heparan sulfate (HS) and chondroitin sulfate (CS) chains, whereas syndecan-2 and syndecan-4 carry only HS chains (15).

Syndecan-1 is a member of heparin sulfate proteoglycan and it is found in epithelial cells and in normal hepatocytes. It is involved in cell growth and differentiation through interaction with growth factors (16).

Elevated serum level of syndecan-1 reported in different malignant tumors and associated with bad prognosis and it correlates to tumor burden, cancer invasiveness, and risk for metastasis. **(**17, 18**)**.

The present study was designed to investigate the potential role of syndecan-1 as a diagnostic, non-invasive biomarker for HCC. Also, to assess its accuracy, sensitivity and specificity as compared with the usual recommended biomarker AFP.

Materials and Methods

This study is cross sectional case–control study. The study included 45 patients with HCC associated with chronic HCV infection. HCC was diagnosed using either guided biopsy or Barcelona non-invasive criteria endorsed by the European Association for the Study of the liver (19). In addition 20 healthy subjects were included as a control group. Informed consent was approved from patients according to the ethical committee of Faculty of Pharmacy, Mansoura University, Mansoura, Egypt.

Patients with HCC not associated with HCV, and other types of malignancy and advanced organ failure were excluded.

Complete medical history was obtained for each subject and complete physical examination was performed. Child-Pugh scoring system was used for staging the severity of liver disease (20).

Following an overnight eight-hour-fast, eight milliliters of whole venous blood samples were withdrawn from each subject; whole EDTA blood was used for complete blood picture, citrated plasma for prothrombin time determination, serum for liver function tests and AFP assays. Liver function tests included determination of alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin, and albumin (ALB). Serum AFP was measured using commercially available ELIZA kits.

Serum syndecan-1 concentration was determined using human syndecan-1 ELISA kit (Boster Biological Technology Co., Ltd., Abingdon OX14 4SE, UK) according to the manufacturer's instructions. A monoclonal antibody from mouse specific for Syndecan-1 has been precoated onto 96-well plates. Standards and test samples were added to the wells; a biotinylated detection polyclonal antibody from goat specific for Syndecan-1 was added subsequently and then followed by washing with the buffer. The color developing agent was catalyzed to produce a blue color-product that changed into yellow color after adding acidic stop solution. The density of the yellow color was proportional to the human Syndecan-1 of sample captured in plate. Optical densities were determined using a microtiter palate reader (Multiscan RC Type 351; Labsystems, Helsinki, Finland) at 450 nm.

Statistical analysis

The results of the study were analyzed by SPSS16. The descriptive statistics, the frequency and percentage, were calculated for qualitative variables. The mean value ± standard deviation

(SD) was used for quantitative variables. For comparison between two groups student t-test was used. Statistical significance was predefined as highly significance ($p< 0.001$) and significant ($P < 0.05$). A receiver operation curve (ROC) analysis was performed to detect cut off point for syndecan-1 with accuracy and percentage of sensitivity and specificity.

Results

The present study included 45 patients with HCC associated with HCV, 82.2% males and HCC patients with mean age 58.7 ±5.5 years (M±SD). They were classified according to Child–Pugh classification into: group A (n=21) 46.7%, group B (n=14) 31.1% and group C (n=10) 22.2%. The majority of patients have tumor size >2.5 cm (80%), with splenomegaly (60%) and associated with ascites (71.1) (Table 1).

Table 1: Demographic and Clinical data of the patients group.

Parameter	HCC Patients (n=45) No. (%)
Sex	
Male	37 (82.2%)
Female	8 (7.8%)
Age	58.7± 5.5
Splenomegaly	27 (60%)
Child-Pugh Score	
A	21(46.7%)
B	14(31.1%)
C	10 (22.2%)
Tumor Size	
≤ 2.5 cm	9 (20%)
>2.5 cm	36 (80%)
Ascites	
Yes	32 (71.1%)
No	13 (28.9%)

n: Number of patients

Laboratory tests for liver function identified in Table 3. There was highly significant elevated serum level of ALT (52.40±21.37 IU/L), and bilirubin (1.7± 0.94mg/dl) ($p<0.001$). Albumin level (3.05±0.6 g/dl) showed highly significant reduction ($p< 0.001$), while, AST reveild non significant elevated serum level ($p>0.05$). Hematological parameters showed highly significant reduction of hemoglobin (5.0± 1.9 g/dl), WBCs (4.3± 2.0cmm $x10^3$) and platelets counts (112.5±16.0 cmm $x10^{3)}$ ($p<0.001$, for each) in patients compared to control subjects. Serum levels of AFP (471.33±116.64 ng/ml) identified highly significant elevated levels in HCC patients ($P<0.001$) compared to control subjects (3.15±0.93ng/ml). Also, syndecan-1 (7480± 101pg/ml) showed marked significant elevated levels in HCC patients ($P<0.001$) compared to control subjects (2223±60 pg /ml) (Table 2, Figure 1).

Statistical comparative study was done between each of syndecan-1 and AFP with some clinical parameters in subgroups of HCC patients (Table 3). There were highly significant elevated syndecan-1 serum levels (7665± 104 pg/ml) in patients with older age than 55 years ($p<0.001$), with tumor size larger than 2.5 cm (7624±122 pg /ml) and with the presence of ascites (7695±97 pg/mg) ($p<0.001$, for each). Moreover, there was significant increase in

syndecan-1 levels in advanced Child-Pugh (C) classification (P<0.001) (Figure 2). Serum AFP levels were significantly affected by tumor size and ascites, only.

ROC curve calculation and analysis result represented in table (4) and figure (4). Best diagnostic performance level represented by Cut-off point concentration for AFP and Syndecan-1 reported 490 ng/ml and 4241 pg/ml, respectively. Area under the curve (AUC) showed a high accuracy for Syndecan-1 (AUC=0.98) compared with AFP (AUC=0.79) and. Syndecan-1 showed higher sensitivity than AFP (99.8%, 85.5%; respectively), while each of them identified moderate specificity (75%, 73%; respectively) in differentiation of patient with HCC. ROC curve statistical results for each of syndecan-1 and AFP at different stage of child-pugh classification represented in figure (5) and table (5). Syndecan-1 reported high accuracy in stage A and C compared with stage B (0.869, 0.814 and 0.675, respectively). Syndecan-1 revealed high specificity at stage A and B (92%, for each) and reached 100% specificity for stage C. On the other hand, AFP reported reduced accuracy than syndecan-1 and none discriminated values in different stages (0.546, 0.548 and 0.506; respectively), with no noticeable specificity difference between stages. Each of syndecan-1 and AFP identified low specificity.

Table 2: Liver functions, hematological parameters, AFP and syndecan-1 levels in HCC patients and control subjects.

Parameters	Control subject (n=20)	HCC Patients (n=45)	*P*
ALT (IU/L)	29.0± 4.2	52.40± 21.37	0.000
AST (IU/L)	41.9±3.2	61.40± 23.21	0.09
ALB (g/dl)	4.07± 0.4	3.05± 0.6	0.000
Bilirubin (mg/dl)	0.82± 0.10	1.7± 0.94	0.000
INR (seconds)	1.2± 0.15	1.29± 0.19	0.2
Hb (g/dl)	13.03± 1.6	5.0± 1.9	0.000
WBCs/cmm	7.47± 2.16 $X10^3$	4.3± 2.0 $X10^3$	0.000
Platelets counts/cmm	234.8± 19.0 $X10^3$	112.5± 16.0$X10^3$	0.000
AFP (ng/ml)	3.15± 0.93	471.33± 116.64	0.000
Syndecan-1 (pg/ml)	2223±60	7480±101	0.000

n: Number of subjects, ALT: alanine aminotransferase, AST: aspartate aminotransferase; ALB: albumin, Hb: Hemogloben, WBCs: White blood cells, AFP: α-Fetoprotien

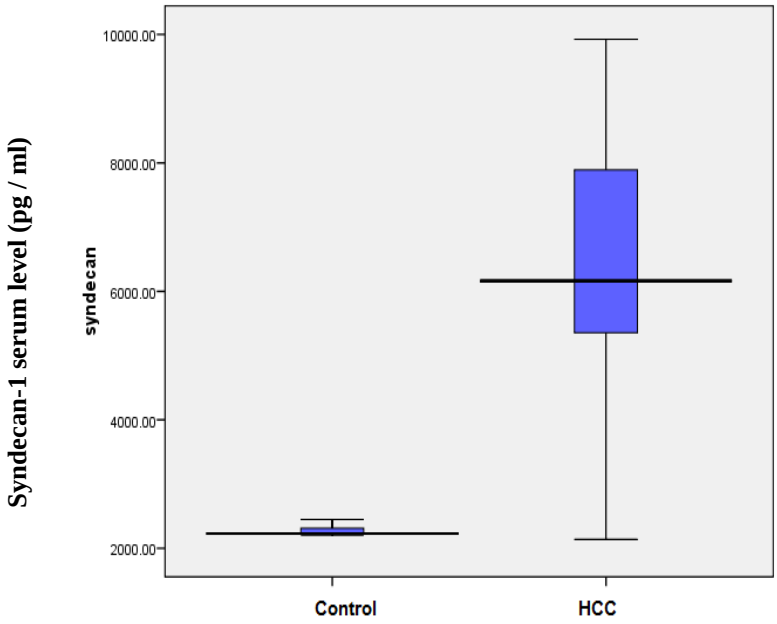

Figure 1: Comparison between syndecan-1 serum levels in patients with HCC and control subjects.

Table 3: Correlation of syndecan-1 serum levels with age, tumor size, Child–Pugh classifications and ascites between HCC patients and control subjects.

Parameter	Syndecan level (pg/ml) (Mean± SD)	*P1*	AFP level (ng/ml) (Mean± SD±)	*P2*
Age				
≤55 years old	6853± 55	0.000	436.0±129.58	0.282
>55 years old	7665± 104		481.43±112.65	
Size of tumor				
≤2.5 cm	6910± 192	0.045	321.11±27.13	0.000
>2.5 cm	7624± 122		508.89±98.39	
Child –Pugh Score				
A	6916± 86	0.000	465.71±140.78	0.942
B	7783± 79		480.03±86.29	
C	8274± 90		471.33±116.64	
Ascites				
Yes	7695± 97	0.023	517.50±102.4	0.000
No	6695± 86		357.69±55.85	

P1, Syndecan level and *p2*, AFP level; each versus different subgroups.

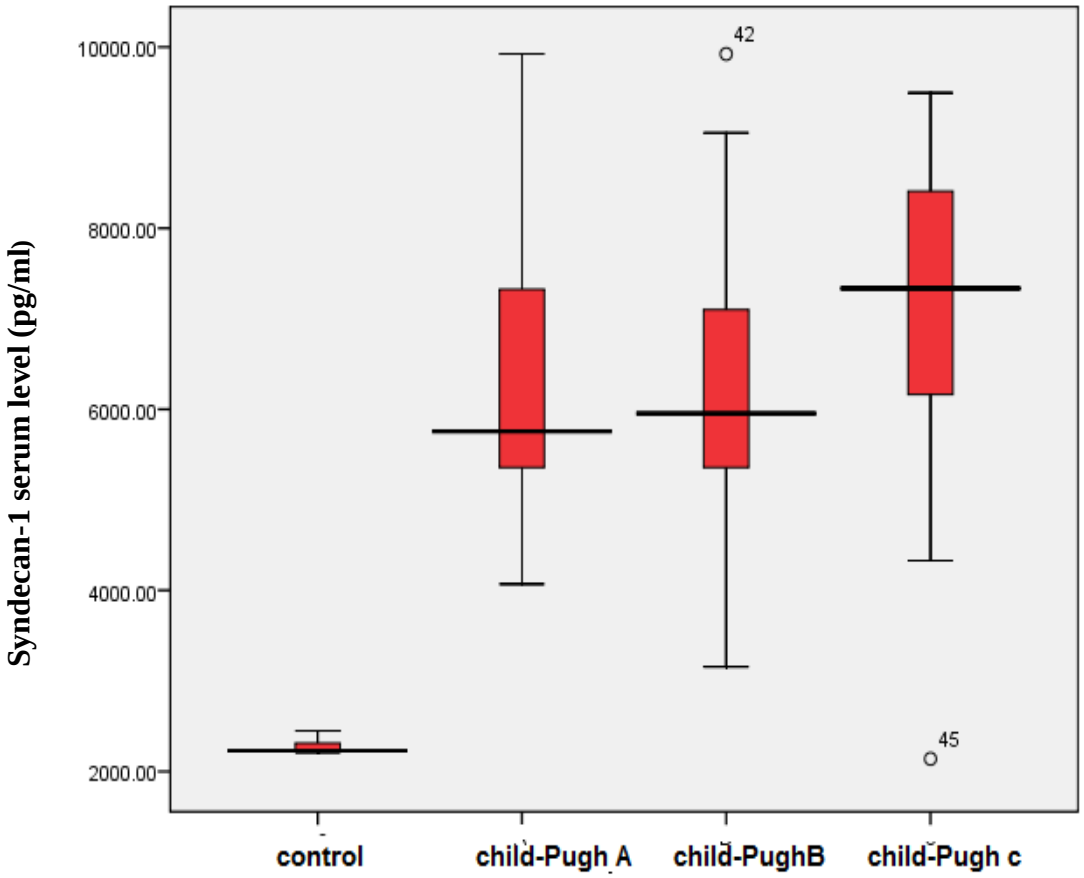

Figure 2: Comparison between Syndecan-1 serum levels in patients with HCC and control subjects according to Child-Pugh classification.

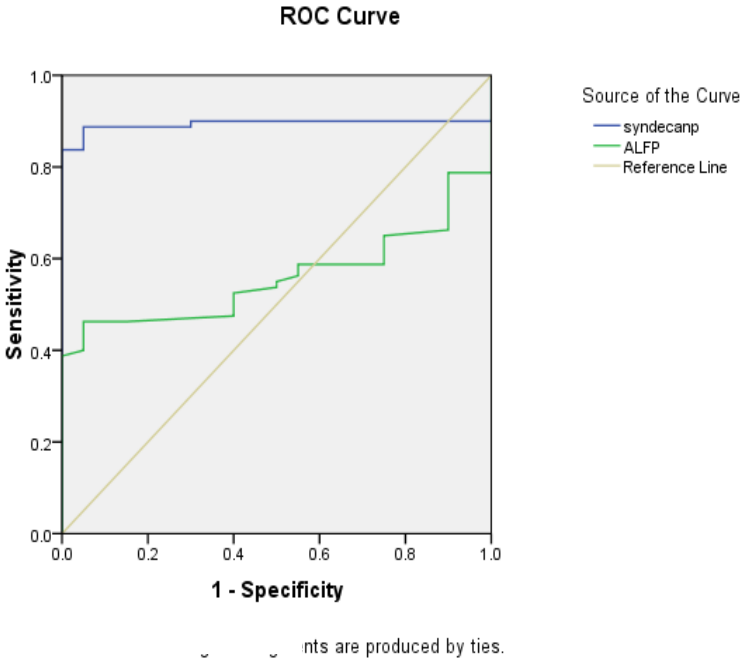

Figure 3: Receiver operating characteristic curve (ROC) of Syndecan-1 and α-fetoprotein (AFP) in patients with HCC.

Table 4: Syndecan-1 and α-fetoprotein (AFP) accuracy cut-off value, sensitivity and specificity in HCC patients.

Marker	Accuracy	Cut-off point	Sensitivity %	Specificity %
AFP	0.79	490 (ng/ml)	85%.	73%
Syndecan-1	0.98	4241 (pg/ml)	99.8%	75%

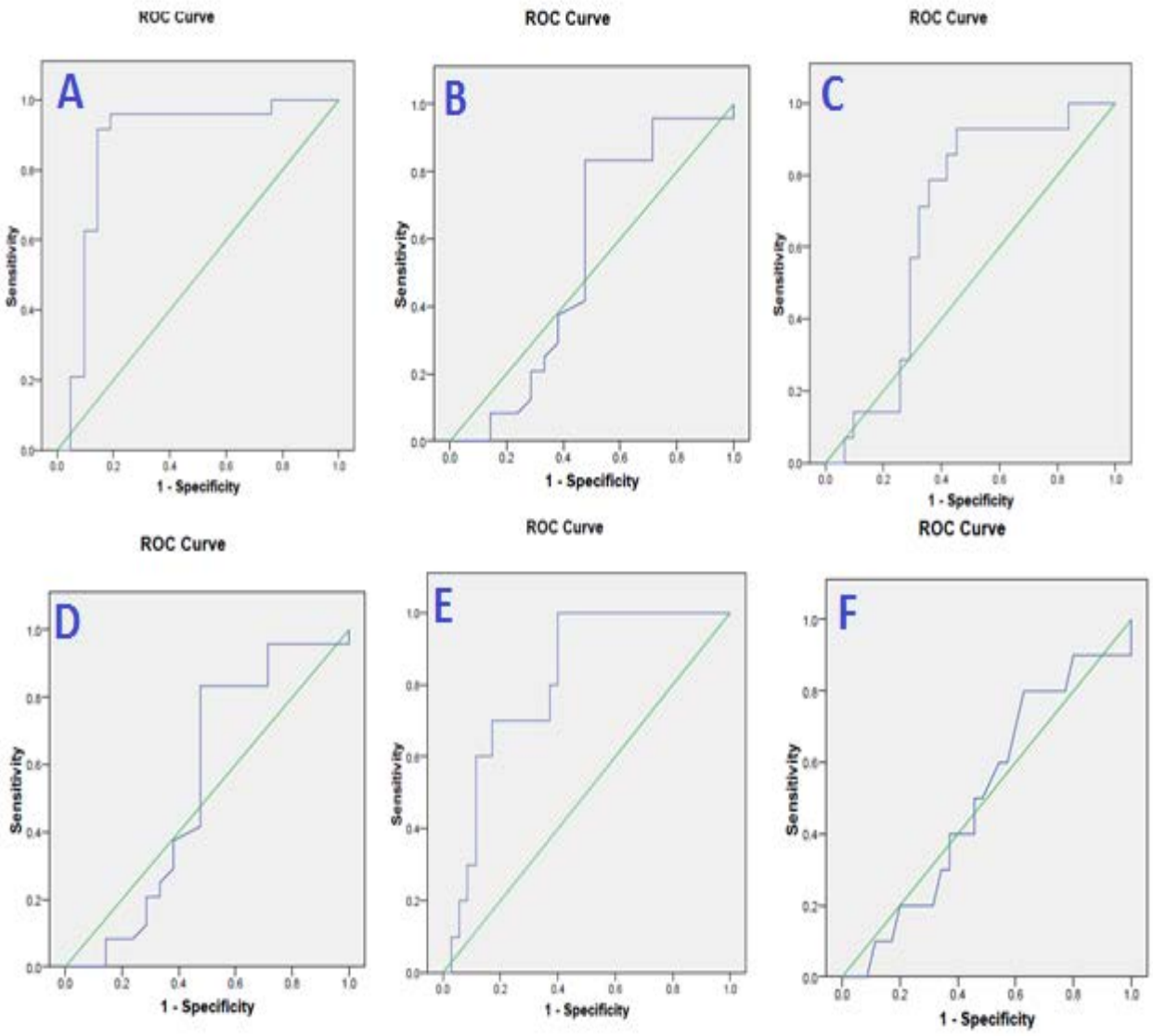

Figure 4: Receiver operating characteristic curve (ROC) of α-fetoprotein (AFP) and Syndecan-1 in different Child-Pugh classification. (A) AFP at stage A. (B) Syndecan-1 at stage A. (C) AFP at stage B. (D) Syndecan-1 at stage B. (E) AFP at stage C. (F) Syndecan-1 at stage C.

Table 5: Receiver operating characterization curve (ROC) for Syndecan and α-fetoprotein (AFP) at different stages of Child-Pugh Classification.

Biomarker	Child-Pugh Score	AUC	Cut-of point	Sensitivity %	Specificity %	Asymptotic 95% CI
Syndecan-1	**A**	0.869	7105 (pg/ml)	92%	15%	0.744-0.994
	B	0.675	7027(pg/ml)	92%	46%	0.515- 0.835
	C	0.814	7158 (pg/ml)	100%	40%	0.686- 0.943
AFP	**A**	0.546	452 (ng/ml)	83%	48%	0.362- 0.729
	B	0.548	452 (ng/ml)	86%	58%	0.382- 0.715
	C	0.506	452 (ng/ml)	80%	63%	0.311-0.700

AUC: Area under the curve = Accuracy. CI: Confidence Interval

Discussion

Hepatitis C virus (HCV) is a member of Hepacivirus genus of the Flaviviridae family. Hepatitis C is a major health problem in Egypt and is a major cause of chronic viral hepatitis worldwide. Hepatitis C virus is a major cause of chronic liver diseases, cirrhosis and HCC (11). The duration of HCV infection is directly related to HCC development (12). HCC incidence in Egypt was doubled in the past ten years as a result of increased prevalence of HCV (10).

Biomarkers detectable in blood, urine, or tissue, as molecular indicators of biological status, can be useful for the clinical management of various disease states. Also, biomarkers could serve as a measurement tool to detect disease presence and progression and to guide more targeted therapy (21, 22).

Our study includes 45 patients with HCC on the top of cirrhosis and with HCV. This is clarified that cirrhosis and HCV are main causes for HCC. This is agree with those studies reported that HCV and HBV infections are the most common risk factors for HCC among Egyptian. In general, about 10-20% of general Egyptian people are infected with HCV (23).

Mostly, HCC is occurs in cirrhotic patients associated with viral infection (24, 25). However, about 10-20% of HCC cases develop in the absence of cirrhosis as a result of the direct oncogenic effect of HBV. In contrast, HCV reported in many studies showed a direct oncogenic action through production of cirrhosis (26-28).

In the present study patient's age were 58.7±5.5 years (Mean±SD) and they were mainly men (82.2%). It is known that male gender is associated with increased risk for development of HCC with HCV. Many hypotheses were defined for this association. Firstly, men have greater incidence of viral hepatitis and alcoholic cirrhosis (29). Secondly, hormonal association involving high serum levels of testosterone is claimed to predispose men to aggravated inflammatory reactions in association with viral hepatitis C or B (30). Older age are usually the age of presentation with HCV related complications either cirrhosis or

HCC (31). The patients were mainly belonging to Child-Pugh classification A. This is an acceptable finding as Child-Pugh classification is related to the degree of liver affection by cirrhosis and not related to the development of HCC (32).

Our results showed marked elevations of serum ALT activities in patients with marked reduction in serum albumin level, and WBCs and platelets. Also HCC patients were older than control subjects. These results agreed with and explained by previous studies reported that patients with chronic hepatic affecting regarding older age of patients with reduced albumin level (33, 34), as the synthetic capacity of the liver is usually affected. Platelets counts were reported as good predictor of chronic liver disorders. This is attributed to the altered production of thrombopoietin (35).

AFP showed significantly marked elevations in HCC patients compared to control subjects. AFP has been used as a useful tumor marker for the detection of HCC; however, it has many limitations in diagnosis of HCC with HCV, as it lacks specificity. Serum AFP values were frequently elevated, even in the absence of HCC in patients with advanced cirrhosis associated with HCV (36). Moreover, its level did no correlate with the tumor size.

In our study, we found a significant increase in serum syndecan-1 concentration in HCC patients compared to control subjects. Syndecan-1 accelerates hepatocyte growth factor (HGF) signals (37). Syndecan-1 binds to a variety of growth and angiogenic factors through heparan sulphate group, this leads to promotion of cellular growth and proliferation (38). Moreover, Syndecan-1 acts as a cell adhesion molecule by interaction with ligands in the extracellular matrix and on cell surfaces (39). Syndecan-1 has been reported to be a modulator of proteolytic activities and chemokine functions *in vivo* thus regulating leucocyte recruitment and tissue remodeling during inflammation and wound repair (40). These functions lead syndecan-1 to act as a regulator molecule in the growth, survival, vascular genesis and metastasis of different types of cancers like myeloma, breast, bladder and colon. However, further studies should be performed to elucidate its putative role in HCC.

The distinguished finding of the present study was the significant association of serum syndecan-1 in HCC patients with older age than 55 years, with larger tumor size more than 2.5 cm, with the presence of ascites and with advanced Child-Pugh classifications.

ROC curve analysis results represented by figure 4 and table 4. Earlier report by Metwaly et al., 2012 (18) support our finding that syndecan-1 was more sensitive than AFP in differentiation of HCC from healthy subjects. Our results revealed (99.8%) sensitivity for syndecan-1 compared with AFP (85%) sensitivity at cut off point's 4242 pg/ml and 430.3 ng/ ml, respectively. Our results is agree with those studies reported that AFP serum level ≥ 400 ng/ml is diagnostic for HCC, although fewer than half of patients may generate levels that high (41). With values of that magnitude, the sensitivity of AFP falls below 45% (42).

Also, ROC curve analysis for both markers depending on Child-Pugh classification represented by Figure 5 and Table 5. Syndecan-1 reported cut-off point 7105, 7027 and 7158 with high sensitivity 92%, 92% and 100%, respectively. On the other hand, AFP revealed reduced sensitivity represented by 83%, 86% and 80% at cut-of points equal for each 425 ng/ml. Also, accuracy values showed higher values with syndican-1 all over the stages compared with AFP accuracy. These results agree with Nadia et al, 2013 (36) and Tundy et al, 2015 (43), where our results reported that syndecan-1 sensitivity was 100% at stage C.

We conclude from this study that syndecan-1is an accurate biomarker in diagnosis and prognosis of HCC. Its levels correlate with tumor size, age of the HCC patients, acietis and Child-Pugh classification of HCC, and thus can aid in direction of therapy. Further studies on larger groups of patients with different stages of chronic liver disease include: fibrotic, cirrhotic and HCC patient are required to validate these results are required to validate these results.

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