Endocan as a Novel Biomarker Versus Alphafetoprotein in Hepatitis C Virus Related Cirrhosis with Hepatocellular Carcinoma

Helmy A., Farag F., Abd El-Fattah N. and Sheta T.

Department of Internal Medicine, Faculty of Medicine, Mansoura University, Mansoura, Egypt.
drtarek_sheta@yahoo.com

Abstract: Background: In Egypt, the prevalence of hepatitis C virus infection is high (about 20%). HCC is the major cause of death in patients with chronic hepatitis C virus infection. HCC is the most common primary liver cancer and the third common cause of cancer related death (14.8%). Alpha Fetoprotein (AFP) is the most commonly used tumor biomarker for the early detection and clinical follow up of patients but its low positive rate, false-positive and false-negative results limits its value in diagnosis of HCC. Endocan is a 50 kDa soluble proteoglycan that is produced and secreted by tumor vascular endothelial cells. Recent studies showed that endocan is overexpressed in HCC tissues and sera and has been associated with tumor progression and poor outcomes. **Aim of the work:** To validate endocan level in HCV cirrhotic patients with or without HCC compared to AFP. **Patients and methods:** Sixty six Egyptian patients with chronic hepatitis C (CHC) were divided into 2 groups and thirty healthy subjects as a control group, measurement of serum endocan and AFP level were done. **Results:** Serum endocan level was significantly high in HCC patients with cutoff point ≥3.59 ng/ml with 100% sensitivity,83% specificity which is superior to AFP level in which cutoff point was ≥14.3 ng/ml with 82%sensitivity and 73% specificity. **Conclusion:** Serum endocan level can be considered a good diagnostic marker in HCC on top of CHC infection as compared to AFP.

[Helmy A., Farag F., Abd El-Fattah N. and Sheta T.. **Endocan as a Novel Biomarker Versus Alphafetoprotein in Hepatitis C Virus Related Cirrhosis with Hepatocellular Carcinoma.** *Life Sci J* 2017;14(8):1-10]. ISSN: 1097-8135 (Print) / ISSN: 2372-613X (Online). http://www.lifesciencesite.com. 1. doi:10.7537/marslsj140817.01.

Keywords: Endocan, Alpha-fetoprotein, Biomarker, Hepatocellular carcinoma

1. Introduction

Worldwide, hepatocellular carcinoma (HCC) is the most common primary liver cancer with over one million new cases annually (**1**) and the third leading cause of cancer related death (**2,3**). HCC is the major cause of death in patients with chronic hepatitis C virus (HCV) infection and responsible for approximately one million deaths each year (**4**).

Egypt has the highest HCV prevalence worldwide (**5**) where about 24% of the people are estimated to carry HCV and more than 50% of blood donors have anti-HCV in some towns **(6,7)**.

The burden of HCC has been increasing in Egypt with a doubling in the incidence rate in the past 10 years **(8,9).** It contributes to 14.8% of all cancer mortality in Egypt **(10)**.

Alpha Fetoprotein (AFP) is the most commonly used tumor biomarker currently available for the early detection and clinical follow up of patients with HCC **(11)**. It has a sensitivity of 41-65% and specificity of 80-94% even when the cut-off value is 20 ng/ml **(12)**.

Internationally, AFP cut-off level of 200 ng/ml is indicative of HCC **(13)**. Also, acute, chronic viral hepatitis as well as patients with cirrhosis caused by hepatitis C may associated with slightly high AFP levels so, its low positive rate, false-positive results and finally false-negative results limits its value in diagnosis of HCC **(11)**.

Endocan, or endothelial specific molecule-1, is a50 kDa soluble proteoglycan that is produced and secreted by activated vascular endothelial tumor cells **(14)**.

Endocan is over expressed at the mRNA level in HCC tissues as shown by multiple recent studies **(15).** Also, over expression of endocan in cancer tissues and sera has been associated with rapid tumor progression and poor outcome **(16).**

There is a significant increase in the density of microvessels in resected tumors, represented by endocan over expression which was prognostic for bad survival (17). Moreover, it was found that increased serum endocan levels and endocan over expression by stromal endothelial cells in HCC tissues were predictive of recurrence after microwave or radiofrequency ablation (18).

Aim of work:

To validate endocan level in HCV cirrhotic patients with or without HCC compared to AFP.

2. Patients and methods

The study was conducted on sixty six Egyptian patients with CHC infection, whom were randomly selected from outpatient clinic of hepatology and early detection of HCC, Specialized Medical Hospital (SMH), Mansoura University, Egypt and 30 healthy volunteers attending for blood donation in the period

between 1/11/2015 and 20/3/2017. This is clinical diagnostic research study to evaluate a diagnostic test and the protocol conforms to the Medical Sciences Ethics Committee of Mansoura Faculty of Medicine and a written informed consent was obtained from each patient enrolled in the study.

The study included both male and female patients, aged between 18-60 years. Patients with other causes of liver cirrhosis, other malignancies (such as multiple myeloma, bladder carcinoma, gastric carcinoma and breast carcinoma), severe co-morbidity that cause sepsis, uncontrolled diabetes mellitus, morbid obesity (BMI ≥40kg/m2) and autoimmune diseases were excluded from the study.

Sample size: Estimated on DSS research which calculate it at level of significance 5% (α error) & power of study 80% and expected size of effect (significant clinical difference) 10%.

For subsequent analysis the cases were divided into 3 groups:

Group 1: Included 30 normal healthy individuals selected from blood donors volunteers in SMH with no history or evidence of any medical disease.

Group 2: Included 33 patients with HCV related hepatitis and cirrhosis attending SMH clinics during their follow up, proved clinically, laboratory & radiologically by abdominal US either compensated or decompensated even with hepatic failure. Then, sub-grouping occurs according to Child-Pugh score.

Group 3: Included 33 patients with HCC on top of HCV related cirrhosis proved radiologically by abdominal US & triphasic abdominal CT. Then, sub-grouping occurs according to Child- Pugh score. Those HCC patients of different stages were selected from patients who attend HCC clinics or admitted in SMH.

All patients were subjected to: History taking, clinical examination and investigations which include: HCV Abs, HBSAg by EIA ***(COBAS Amplicore, Germany)***. serum creatinine, serum albumin, serum bilirubin, Prothrombin time, INR ratio, ALT, AST by automated biochemistry analyzer ***(Cobas Integra 400, Roch diagnostics).*** complete blood count was done on Sysmix KX-21 automatic cell counter ***(Japan)***.

Serum alpha feto protein was measured by ELISA (enzyme-linked immunosorbent assay) kit based on a direct solid stage sandwich method. Measurement of serum endocan was done using an ELISA kit (Endo Mark H1; Lunginnovs.a.s., Lille, France) according to the instructions provided by the manufacturer.

Liver function status (for cirrhosis group) was assessed according to Child-Pugh classification. Imaging studies done were abdominal US for all subjects, triphasic CT abdomen for HCC group diagnosis and fibroscan when cirrhosis is not apparently diagnosed clinically and radiologically.

Sample collection: a sample of 5 cc of blood was withdrawn from each CHC patient and control group. Serum was separated from whole blood within 30 min, made two aliquots and was stored at - 80 º c for further analysis.

Statistical analysis

The collected data were coded, processed & analysed using SPSS program for windows. The level of significance was considered at 5% ($P \leq 0.05$). Quantitative data were represented as mean± SD. We used The Mann–Whitney U or the Kruskal–Wallis tests to compare continuous variables. Also, we used Fisher's exact probability test to compare qualitative variables. The correlation between each of child classification and fibroscan as an outcome and tumour markers as predictors was tested using Spearman's correlation analysis. ROC curve analysis was used to differentiate between HCC and HCV cirrhotic patients, with the best cut-off value based on the Youden index.

3. Results

The present study was conducted on sixty sex Egyptian patients with CHC infection (anti-HCV seropositive and detectable HCV-RNA) and thirty healthy subjects as control. The included patients had age of about55±7.1 years and control subjects aged 39 ±9.5 years. In HCC group there are 27 M (81.8%) and 6(18.2%) female and in chronic liver disease (CLD) group 16 M (48.5%) and 17 F (51.5%) but in health control group the incidence was equal as shown in table (1).

There was significant difference between all studied groups in age, gender, antibilharzial drugs intake prevalence, all biochemical data including endocan, AFP, sonographic findings of liver, spleen, ascites, child classification and fibroscan (all p value<0.05).

Thefrequencyofage, ALT, AST,s.albumin, s.bilirubin, INR, s.creatinine, tumour markers and fibroscan level were found to be significantly higher in cirrhotic group when compared with healthy group (all p value <0.05). Also, the frequency of antibilharzial drug intake, HB, WBCs, Platelets count, encephalopathy, radiographic findings of spleen & ascites & portal vein were found to be significantly high in cirrhotic group when compared with healthy group (all p value <0.05) table (1).

Table (1): Characteristics of studied groups

Characteristic	HCC	CLD	Control	P	P1	P2	P3
Gender N, (%) M F	27(81.8%) 6(18.2%)	16(48.5%) 17(51.5)	15(50%) 15(50%)	0.008	<0.001	0.9	<0.001
Age (years)	55±7.1	55± 7.9	39± 9.5	<0.001	0.99	<0.001	<0.001
Residence N, (%) Urban Rural	25 (75.8) 8(24.2)	20(60.6%) 13(39.4%)	19(63.3%) 11(36.7%)	0.38	0.19	0.28	0.28
Hypertension N, (%) Yes No	10(30.3%) 23(69.7%)	7(21.2%) 26(78.8%)	8(26.7%) 22(73.3%)	0.7	0.4	0.61	0.75
Antibilharzial drugs N, (%) NO Oral drugs IV tartar emetic	9(27.3%) 7(21.2%) 7(51.5%)	11(33.3%) 9(27.3%) 13(39.9%)	18(60%) 9(30%) 3(10%)	0.0009	0.61	0.017	<0.001
Encephalopathy N, (%) No Grade I & II Grade III & VI	27(81.8%) 5(15.2%) 1(3%)	29(87.9%) 4(12.1%) 0(0%)	30(100%) 0(0%) 0(0%)	0.1	0.73	0.03	____
Haemoglobin (gm/dl)	11.8± 1.1	11.5± 1.8	12.7± 1.4	<0.001	0.64	<0.001	0.036
WBCs count (	4.2± 1.2	3.8± 1.1	5.1 ±1.6	0.01	0.75	0.01	0.06
Platelets count	86(69_137)	91(79_128)	221(153_294)	<0.001	0.94	<0.001	<0.001
ALT (IU\ml)	45(25-68)	45(35-68)	33(23-43)	<0.001	0.51	<0.001	0.01
AST (IU\ml)	32(25-57)	42(39-70)	30(23-34)	<0.001	0.02	<0.001	0.02
Bilirubin (mg\dl)	1.5±0.5	1.3± 0.4	0.67± 0.2	<0.001	0.25	<0.001	<0.001
s. albumin (gm/dl)	3.4± 0.5	3.3±0.5	4 ±0.6	<0.01	0.91	<0.001	<0.001
INR	1.3± 0.2	1.4 ±0.3	1 ± 0.1	<0.001	0.05	<0.001	<0.001
s. creatinine (mg\dl)	0.9 ± 0.2	0.9±0.2	0.6± 0.2	<0.001	0.62	<0.001	<0.001
Alpha fetoprotein (ng/ml)	162(24.5_943.5)	8.5(7-47.5)	7(5-10.5)	<0.001	<0.001	<0.001	<0.001
Endocan (ng\ml)	7.4 ± 2.2	3.7± 1.2	1.3± 0.2	<0.001	<0.001	<0.001	<0.001
Liver (u/s) N, (%) Normal Average cirrhotic Enlarged cirrhotic Shrunken cirrhotic	0(0%) 13(39.4%) 7(21.2%) 13(39.4%)	1(3%) 19(57.6%) 6(18.2%) 7(21.2%)	30(100%) 0(0%) 0(0%) 0(0%)	<0.001	0.23	0.01	___
Hepatic focal lesion (u\s) N, (%) No FL Single<2cm Single>2cm and<5cm or ≤3FL each<5cm Single FL>5cm Multiple >3lesions	6(18.2%) 5(15.2%) 2(6.1%) 10(30.3%) 10(30.3%)	33(100%) 0(0%) 0(0%) 0(0%) 0(0%)	30(100%) 0(0%) 0(0%) 0(0%) 0(0%)	____	___	___	___
Hepatic focal lesion (CT) N, (%) No FL Single<2cm Single>2cm and<5cm or ≤3FL each<5cm Single FL>5cm Multiple >3lesions	0(0%) 2(6.1%) 5(15.2%) 10(30.3%) 16(48.5%)	33(100%) 0(0%) 0(0%) 0(0%) 0(0%)	30(100%) 0(0%) 0(0%) 0(0%) 0(0%)	___	___	___	___
Portal vein (U/S) N, (%) Normal Dilated Thrombosed	1(3%) 27(81.8%) 5(15.2%)	7(21.2%) 23(69.7%) 3(9.1%)	30(100%) 0(0%) 0(0%)	<0.001	0.08	0.02	___
Spleen (u\s) N, (%) Normal Mild splenomegaly Moderate splenomegaly Huge splenomegaly Splenectomy	0(0%) 10(30.3%) 16(48.5%) 4(12.1%) 3(9.1%)	0(0%) 14(42.4%) 12(36.4%) 6(18.2%) 1(3%)	30(100%) 0(0%) 0(0%) 0(0%) 0(0%)	<0.001	0.49	__	___

Characteristic	HCC	CLD	Control	P	P1	P2	P3
Ascites (u\s) N, (%) No Mild to moderate Moderate to sever	23(69.7%) 9(27.3%) 1(3%)	21(63.6%) 8(24.2%) 4(12.1%)	30(100%) 0(0%) 0(0%)	<.001	0.52	0.01	—
Lymph nodes N, (%) Normal Enlarged	28(84.8%) 5(15.2%)	33(100%) 0(0%)	30(100%) 0(0%)	—	—	—	—
Metastasis N, (%) No Yes	28(84.8%) 515.2%)	33(100%) 0(0%)	30(100%) 0(0%)	——	——	——	——
Child classification N, (%) Normal A B C	0(0%) 23(69.7%) 9(27.3%) 1(3%)	0(0%) 22(66.7%) 8(24.2%) 3(9.1%)	30(100%) 0(0%) 0(0%) 0(0%)	<0.001	0.71	——	——
Fibroscan N, (%) F0 F1 F2 F3 F4	0(0%) 0(0%) 1(3%) 1(3%) 31(94%)	0(0%) 0(0%) 2(6.1%) 11(33.3%) 20(60.6%)	10(33.3%) 16(53.3%) 4(13.3%) 0(0%) 0(0%)	<0.001	<0.001	<0.001	<0.001

Data presented as mean ±SD or median IQR SD: standard deviation P: probability.
P: significance between all groups P1: significance between HCC group and cirrhotic group.
P2: significance between cirrhotic group and healthy group. P3: significance between HCC group and healthy group.

In univariate analysis, there was a significant positive association between each of endocan & AFP as a predictor and the prevalence of HCC as an outcome (both OR >1, both p value<0.05) as shown in table (2).

Table (2): Association between HCC and tumor marker (simple logistic regression analysis).

Tumor marker	OR	CI of **OR**	*P* value
Endocan	2.3	1.7-3.1	<0.001
AFP	1.02	1.01-1.03	0.001

OD: odds ratio CI 95%: confidence interval.

There was a significant positive association between each of serum endocan & AFP and child classification of studied groups (both *p* value<0.05) table (3).

Table (3): Association of serum endocan and AFP levels with different child classification.

Character	Child A (n=75)	Child B & C (n=21)	*P* value
Endocan (ng\ml)	2.8(1.34-6.6)	4.35(3-8.2)	0.016
AFP (ng/ml)	10(6-40.5)	66(9.3-289)	0.001

In multiple logistic regression analysis, there was a significant positive association of endocan as and prevalence of HCC after adjustment of AFP (OR >1, p value <0.05) and there was no significant association of AFP and prevalence of HCC after adjustment of endocan (p value >0.05) table (4).

Table (4): Association between HCC and tumor marker (multiple logistic regression analysis).

Tumor marker	OR	CI of OR	P value
Endocan	2.2	1.6 - 3.1	<0.001
AFP	1.02	.99 - 1.04	0.13

Roc curve analysis for serum HCC markers showed that sensitivity, specificity, negative predictive value, positive predictive value of prediction of HCC by endocan level in which cutoff point ≥3.59 ng/ml were 100%, 83%, 100%, 75% respectively.

The sensitivity, specificity, negative predictive value, positive predictive value of prediction of HCC

by AFP level in which cutoff point ≥14.3 ng/ml were 82%, 73%, 88%, 61% respectively.

The sensitivity, specificity, negative predictive value, positive predictive value of prediction of HCC by both endocan and AFP level in which cutoff point ≥3.59 ng/ml, ≥14.3 ng/ml respectively was 82%, 90.5%, 82%, 90.5% respectively.

Table (5): prediction of HCC by tumour markers using Roc curve analysis.

Tumor marker	AUC (95% CI)	P-value	sensitivity	Specificity	Negative predictive value	Positive predictive value	Accuracy	Cut off point
Endocan	0.95(0.92-.99)	<0.001	100%	83%	100%	75%	88.5%	≥3.59
			55%	100%	80%	100%	84%	≥7.85
AFP	.83(o.72-0.93)	<0.001	82%	73%	88%	61%	76%	≥14.3
			51.5%	100%	79%	100%	83%	≥155
Both markers are positive			82%	90.5%	82%	90.5%	87.5%	Endocan≥3.59 and AFP ≥14.3

Using spearman correlation, there was strong correlation between child classification as an outcome and tumour markers as predictors (p value <0.001). There was also strong correlation between fibroscan as an outcome and tumour markers as predictors (p value <0.001) table (6).

There was no significant association between both tumour markers and tumour size using univariate and multivariate analysis table (7,8).

Table (6): Spearman correlation between each of child classification & fibroscan of studied groups and tumour markers.

	Child classification		Fibroscan	
	R	P	R	P
Endocan	0.654	<0.001	0.812	<0.001
AFP	0.487	<0.001	0.59	<0.001

Table (7): Simple logistic regression analysis using tumour markers as a predictors and tumour size as an outcome (fit cases for intervention coded 0 & unfit cases for intervention coded 1).

	OR	CI of OR	P value
Endocan	0.81	0.53-1.25	0.346
AFP	1.003	0.99-1.008	0.161

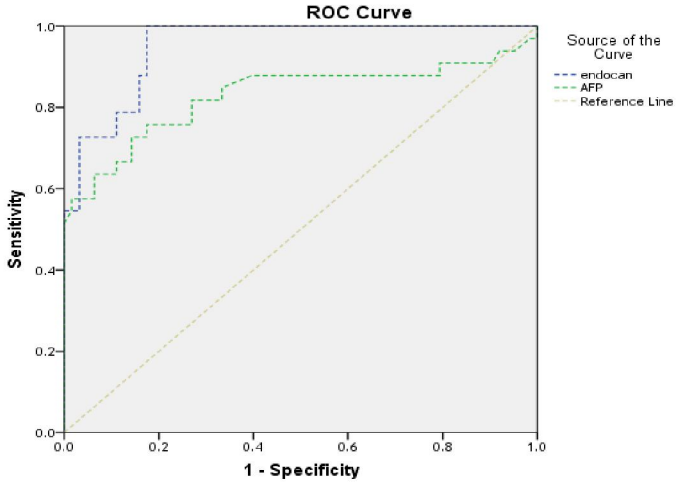

Figure (1): Prediction of HCC by tumour markers using ROC curve analysis.

Table (8): Multiple logistic regression analysis using tumour markers as apredictors and tumour size as an outcome (multivariate analysis).

	OR	CI of OR	P value
Endocan	0.859	.555-1.329	0.499
AFP	1.003	0.998-1.008	0.176

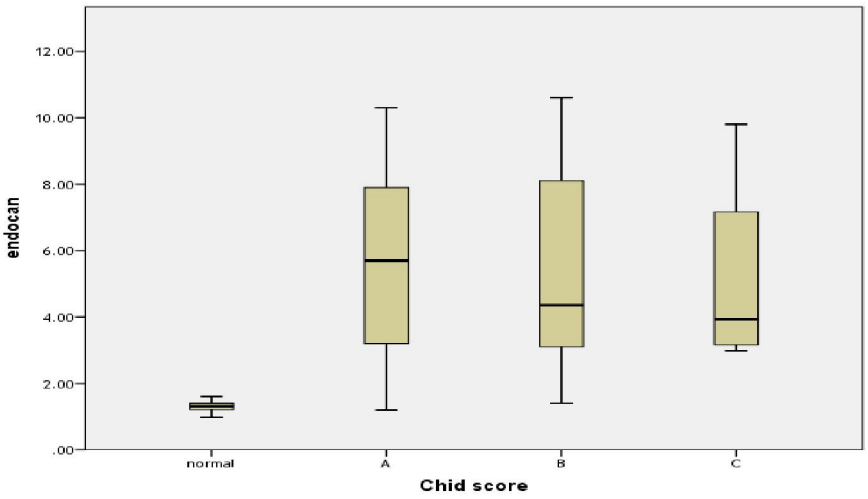

Figure (2): Association between endocan level and child score of studied groups.

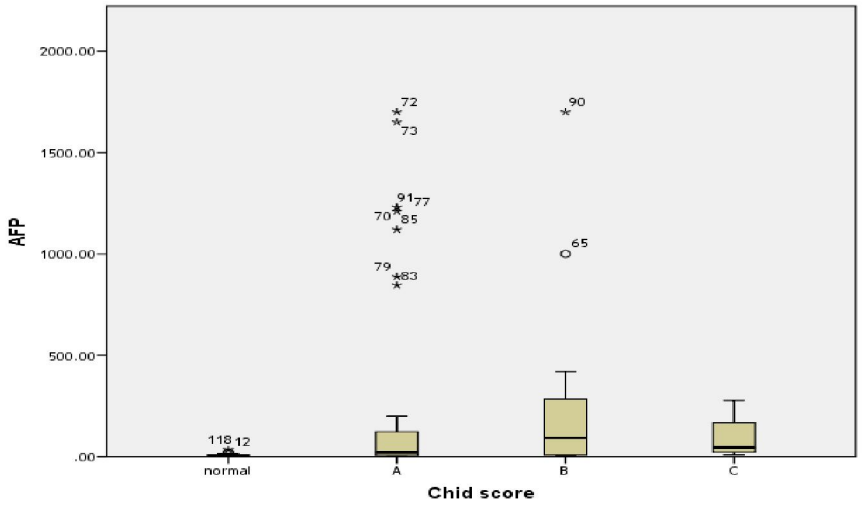

Figure (3): Association between AFP level and child score of studied groups.

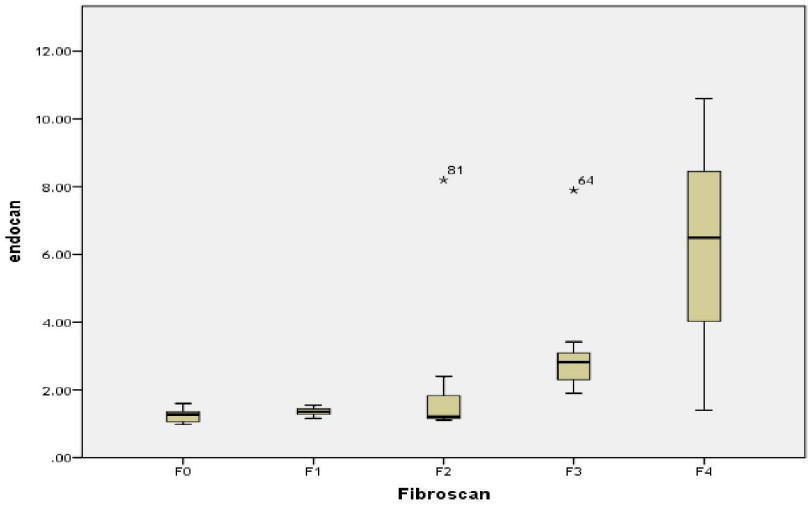

Figure (4): Association between endocan level and fibroscan of studied groups.

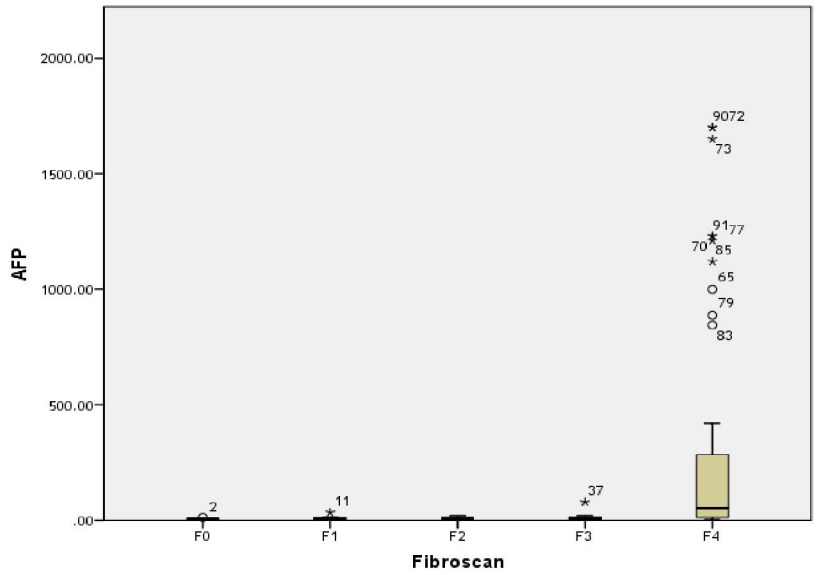

Figure (5): Association between AFP level and fibroscan of studied groups.

4. Discussion

HCC is one of the most common cancers in the world and particularly in Egypt **(19)**. Globally, it is the third leading cause of cancer related death **(2,3)**. HCC is the major cause of death in patients with chronic hepatitis C (HCV) virus infection and responsible for approximately one million deaths each year **(4)**.

Egypt has the highest worldwide prevalence with 9% countrywide and up to 50% in certain rural areas (lower Egypt governorates) due to specific modes of infection **(20)**.

During the past years, several studies have been published concerning different HCC risk factors and burden. The reason for that effort is the increasing number of HCC patients worldwide especially in Egypt which has the highest worldwide prevalence, also absence of HCV vaccine. So, much efforts done to relieve medical, social, and economic costs.

Our study focused on trying to detect new biomarkers rather than AFP for diagnosis of HCC as AFP not a useful diagnostic and prognostic marker for HCC that is proved by **Stefaniuk *et al.* (21)** as they found that 25%-30% of the diagnosed HCC patients have a normal AFP level (< 20 µg/L) and 40%-50% of HCC patients have a low serum AFP level (> 20 µg/L - < 400 µg/L).

There was no significant association between AFP and tumor size using univariate and multivariate analysis in our study in difference with a lot of authors who proved that AFP increase with the size of the tumor **(22) (23)** may be due to different sample size and multiple HCC etiologies.

Our study showed increased ALT in HCC group which may be explained by viral etiology **(24)**, not all HCCs secrete AFP **(25).**

In our study, we found that the median serum level of AFP was highly significantly different among the three groups (p value <0.001). A marked increase was shown in the HCC group, while a slight increase was revealed in the HCV cirrhotic group, this is in agreement with **Soresi *et al.* (26)** who proved that the mean serum level of AFP was highly significant different among the three groups (HCC, LC and control groups). A marked increase was shown in the HCC group, while a slight increase was seen the cirrhotic group.

In our study, we found that the cutoff of AFP was >14.3 giving sensitivity 82% and specificity 73% (table5). Some researchers **(26)** found that the best cut-off value of AFP has been reported to be 30ng/mL (sensitivity of 65%, specificity of 89%). This slight difference may be due to use of healthy persons as a control but they used cirrhotic non hepatitis group persons as a control group.

Forner *et al.* (27) found that if cut-off was 20ng/ml it gives sensitivity 60% only but in our study the sensitivity was 87.5% when cut-off 19.5ng/ml.

Zachary *et al.* (28) revealed a significant elevation in AFP in the HCC group compared to the cirrhotic and normal control groups in agreement with our study.

In our study we found that the median level of endocan in healthy group is 1.3±0.2 ng/ml in agreement with a previous study done **(29)** (1.14 ng/ml) which was slightly higher than other studies such as **(30) (31) (32) (33)** which report that the serum level was (0.3-0.77ng/ml)**.**

In our study we found the cutoff level between cirrhotic and HCC group was ≥3.59 ng\ml with AUC 0.95, sensitivity 100%, specificity 83%, Accuracy

88.5%, NPV 100% and PPV 75% in agreement with a previous study **(34)** in which cutoff level is slightly lower 1.95ng/ml with AUC 0.823 sensitivity 79%, specificity 71%, NPV 80% and PPV 60%.

Our findings showed that endocan level is higher in HCC group versus cirrhotic group which was similar with two previous studies **(35) (36).**

On univariate analysis for predictive value of endocan and AFP in HCC we found that there was a significant positive association between each of endocan and AFP as a predictor of HCC (both OR>1 & both P value<0.05) in agreement with a previous study **(29) (P value 0.03).**

In multivariate analysis there was a significant positive association of endocan as a predictor and prevalence of HCC as an outcome after adjustment of AFP (OR >1, p value <0.001) in agreement with a previous study **(33)** (P value <0.05) and in difference with another study **(29)** (P value 0.364) may be due to insufficient sample size and different baseline characteristics of studied groups. There was no significant association of AFP as a predictor and prevalence of outcome of HCC after adjustment of endocan (p value >0.13).

Roc curve analysis for endocan and AFP level in HCC showed that the sensitivity, specificity, negative predictive value, positive predictive value of prediction of HCC by endocan level in which cutoff point ≥3.59 ng/ml were 100%,83%,100%,75% respectively in agreement with a previous study done by **(37)** in which the cutoff level ≥3.59 ng/ml but with 54.7% sensitivity and 86.8% specificity. The sensitivity, specificity, negative predictive value and positive predictive value of prediction of HCC by AFP level in which cutoff point ≥14.3 ng/ml were 82%, 73%, 88%, 61% respectively. The sensitivity, specificity, negative predictive value, positive predictive value of prediction of HCC by both endocan and AFP level in which cutoff point ≥3.49 ng/ml, ≥14.3 ng/ml respectively were 82%, 90.5%, 82%, 90.5% respectively.

In our study there was strong correlation between serum endocan level and child classification of studied groups (p value <0.001). We also demonstrated that in both HCV cirrhotic and HCC patients the increased serum endocan levels were associated with poor hepatic function. Also, serum endocan levels were increased in HCC versus HCV cirrhotic patients regardless the child score. We found that the median endocan level in child A group was 2.8ng/ml while in child B & C group was 4.38ng/ml which is slightly lower than a previous study **(29)** in which the endocan is 3.38,5.48 ng/ml respectively. This is evident in our study as we found that there is high significance between endocan level and child classification of both groups (p value =0.016) in agreement with two previous studies **(38) (29)** (p value 0.05).

There was strong correlation between serum endocan level and liver histology by fibroscan (p value <0.001) which is similar with a previous study **(38).**

Another group of researchers **(39)** found that there is no relation between serum endocan and sex of studied patients (p value >0.05) in different with our study which showed significant difference between males and females (p value =0.03) due to different sample size and different baseline characteristics.

As regard sex of studied groups, we found a highly significant association between all groups with male predominance (P value<0.001) in different with a lot of studies **(29) (34).** This male predominance is due to high incidence of IV tartar emetic injection among males (51.5% in HCC group,39.9% in CLD group) with increased incidence of HCV infection**.**

Our study showed that there is no significant association between AFP and sex of the patients in agreement with a lot of previous studies **(34) (38) (40) (41) (42).**

Our study found that there is high significance as regard age of studied groups as all (P value<0.001). Also, we found a high significant association between age of cirrhotic & healthy group and HCC group & healthy group in agreement with a previous study **(34)** as HCC need a long time for its pathogenesis as it occur on top of cirrhotic liver and cirrhosis need also from 10-20 years to occur on top of healthy liver so it occurs mostly in elder age group, this is in contrast to HBV in which HCC may occur on top of healthy liver due to its DNA nature so we found HCC in younger age group in chronic HBV infection. HCC may occur in young age in CHC infection may be due to combination of multiple factors such as combined infection of CHB or HIV infection and Aflatoxin exposure in badly stored cereals.

Our study proposed that serum endocan level may be a good tool as a diagnostic novel biomarker in HCC patients. Also, the use of both serum levels of endocan and AFP can lead to better screening and early detection of these patients which will help in arranging therapeutic modalities. Further large studies may be needed in order to validate serum endocan as a predictive biomarker after different treatment strategies.

In Conclusion

We can consider serum endocan level as good diagnostic marker in HCC patients however more researches are needed to confirm these results and its utility in diagnosis and follow up of HCC patients with or without intervention.

Acknowledgements:

We thank all the clinicians and patients for their cooperation in the study.

Conflicts of interest:

There are no conflicts of interest.

Corresponding author:
Name: Dr. Tarek Sheta, MD
Address: Hepatology and Gastroenterology Unit, Lecturer of Internal Medicine, Internal Medicine Department, Faculty of Medicine, Mansoura University, Egypt.
E-mail: drtarek_sheta@yahoo.com

References:

1. WalyRaphael S, Yangde Z, & Yu Xiang C. Hepatocellular Carcinoma: Focus on Different Aspects of Management. ISRN Oncol 2012; 421673.
2. Murata S, Mine T, Ueda T, et al. Transcatheter Arterial chemoembolization Based on Hepatic Hemodynamics for Hepatocellular Carcinoma. The Scientific World Journal 2013; (479805):1-8.
3. Venepalli NK & Goff L. Targeting the HGF-cMET Axis in Hepatocellular Carcinoma. International Journal of Hepatology 2013; (341636):1-11.
4. Andrade LJ, Junior AD, Melo RC, et al. Association Between Hepatitis C and Hepatocellular Carcinoma. J Glob Infect Dis 2009; 1(1):33–7.
5. Omar A, Abou-Alfa GK, Khairt A, et al. Risk factors for developing hepatocellular carcinoma in Egypt. Chin Clin Oncol 2013; 2(4):43.
6. *Raphael SW, Yangde Z & Yu Xiang C.* Hepatocellular Carcinoma: Focus on Different Aspects of Management. ISRN Oncology 2012; 421673.
7. Schiefelbein E, Zekri AR, Newton DW, et al. Hepatitis C virus and other risk factors in hepatocellular carcinoma. Actavirologica 2012; 56:235–240.
8. Attalla SM, El-Azab SM, El-Bakary AA, et al. is aflatoxinb A is acommon risk factorfor hepatocellular carcinoma Mansoura J. Forensic Med. Clin. Toxicol. 2009; 17(2):15-25.
9. Azmy AM, Nasr KE, Gobran NS, et al. Gemcitabine Plus Carboplatin in Patients with Advanced Hepatocellular Carcinoma (HCC): Results of a Phase II Study. Open Access Scientific Reports 2012; 1(7):1-4.
10. Radwan NA & Ahmed NS. (2012): The diagnostic value of arginase-1 immunostaining in differentiating hepatocellular carcinoma from metastatic carcinoma and cholangiocarcinoma as compared to Hep Par-1. Diagnostic Pathology 2012; 7:149.
11. Behne T & Copur S. Biomarkers for Hepatocellular Carcinoma. International Journal of Hepatology 2012; 859076.
12. Debruyne EN & Delanghe JR. Diagnosing and monitoring hepatocellular carcinoma with alpha-fetoprotein: new aspects and applications. Clin Chim Acta. 2008 Sep; 395(1-2):19-26.
13. El-Zayadi A, Badran HM, Barakat EMF et al. Hepatocellular carcinoma in Egypt: A single center study over a decade. World J Gastroenterol 2005; 11(33):5193-8.
14. Abid M R, Yi X, Yano K, Shih S C, Aird W C. Vascular endocan is preferentially expressed in tumor endothelium. Microvasc Res. 2006; 72: 136-45.
15. Kang Y H, Ji N Y, Lee C I, et al. ESM-1 silencing decreased cell survival, migration, and invasion and modulated cell cycle progression in hepatocellular carcinoma. Amino Acids.2011; 40: 1003-13.
16. Cornelius A, Cortet-Rudelli C, Assaker R, et al. Endothelial expression of endocan is strongly associated with tumor progression in pituitary adenoma. Brain Pathol. 2012; 22: 757-64.
17. Huang G W, Tao Y M, Ding X. Endocan expression correlated with poor survival in human hepatocellular carcinoma. Dig Dis Sci. 2009; 54: 389-94.
18. *Ziol M, Sutton A, Calderaro J, et al.* ESM-1 Expression in Stromal Cells is Predictive for Recurrence after Radiofrequency Ablation in Early Hepatocellular Carcinoma. J Hepatol. 2013.
19. Khalid O, Bouneva I. (2011): Management of hepatocellular carcinoma: treatment options and indications for orthotopic liver transplantation. Mo Med; 108:264–268.
20. *Kamal SM, Nasser IA. (2008):* Hepatitis C genotype 4: what we know and what wedon't yet know. Hepatology;47:1371–1383.
21. Stefaniuk P, Cianciara J, Wiercinska-Drapalo A. (2010): Present and future possibilities for early diagnosis of hepatocellular carcinoma. World J Gastroenterol; 16:418–424.
22. bbasi A, Bhutto A, Butt N & Munir SM. (2012): Correlation of serum alpha fetoprotein and tumor size in hepatocellular carcinoma. J Pak Med Assoc; 62(1):33-6.
23. *Zhao Y, Ju Q & Li G.* Tumor markers for hepatocellular carcinoma (Review). MOLECULAR AND CLINICAL ONCOLOGY 2013; 1:593-8.

24. *Al Swaff R. (2012)*: Correlation between alanine aminotransferase level, HCV-RNA titer and fibrosis stage in chronic HCV genotype 4 infection. Egyptian Journal of Medical Human Genetics Volume13, Issue 2, Pages 207–212.
25. Debruyne EH, Delanghe JR. (2008): Diagnosing and monitoring hepatocellular carcinoma with alpha-fetoprotein: new aspects and applications. Clin Chim Acta; 395:19–26.
26. Soresi M, Magliarisi C, Campagna P, Leto G, Bonfissuto G, Riili A, Carroccio A, Sesti R, Tripi S, Montalto G. (2003): Usefulness of alpha-fetoprotein in the diagnosis of hepatocellular carcinoma. Anticancer Res; 23(2): 1747-53.
27. Forner A1, Llovet JM, Bruix J(2012): role of AFP in HCC diagnosis 2012 Mar 31;379(9822):1245-55. doi: 10.1016/S0140-6736(11)61347-0. Epub 2012 Feb 20.
28. Zachary, Khodeer S, Ezzat H, Abdel Malak C. (2012): Impact of PIVKA-II in diagnosis of hepatocellular carcinoma. International Conference on Ecological, Environmental and Biology Sciences (ICEEBS' 2012), Jan. 7–8. 2012 Dubai.
29. Kazuaki Ozaki, Nobuyuki Toshikuni, Joseph George, Takahiro Minato, Yasuhiro Matsue1, Tomiyasu Arisawa and Mikihiro Tsutsumi (2014): Journal of Cancer 5(3): 221-230. doi: 10.7150/jca.7691.
30. Behne T & Copur S: Biomarkers for Hepatocellular Carcinoma. International Journal of Hepatology 2012; 859076.
31. Grigoriu B D, Depontieu F, Scherpereel A, et al (2006): Endocan expression and relationship with survival in human non-small cell lung cancer. Clin Cancer Res. 2006; 12: 4575-82.
32. Leroy X, Aubert S, Zini L, et al (2013): Vascular endocan (ESM-1) is markedly overexpressed in clear cell renal cell carcinoma. Histopathology; 56: 180-7.
33. Roudnicky F, Poyet C, Wild P, et al (2013): Endocan is upregulated on tumor vessels in invasive bladder cancer where it mediates VEGF-A-induced angiogenesis. Cancer Res. 2013; 73: 1097-106.
34. Duran Tok, Fuat Ekiz, Omer Basar, Sahin Coban, Gulfer Ozturk (2014): Int J Clin Exp Med 2014;7(7):1802-1807.
35. Kang YH, Ji NY, Lee CI, Lee HG, Kim JW, Yeom YI, et al. (2011): ESM-1 silencing decreased cell survival, migration, and invasion and modulated cell cycl progression in hepatocellular carcinoma. Amino Acids;40:1003-13.
36. Nault J C, Guyot E, Laguillier C, et al (2013): Serum Proteoglycans as Prognostic Biomarkers of Hepatocellular Carcinoma in Patients with Alcoholic Cirrhosis. Cancer Epidemiol Biomarkers Prev. 2013; 22: 1343-52.
37. Nobuyuki Toshikuni2*, Joseph George1, Takahiro Minato1, Yasuhiro Matsue1, Tomiyasu Arisawa2 an Mikihiro Tsutsumi: Journalof Cancer2014; 5(3): 221-230. doi: 10.7150/jca.7691.
38. Nasser Bayati, M.D., Ann L. Silverman, M.D., and Stuart C. Gordon, M.D (1998): Vol. 93, No. 12, 1998 Copyright © 1998 by Am. Coll. of Gastroenterology ISSN 0002-9270/98/$19.00 Published by Elsevier Science Inc. PII S0002-9270(98)00584-X.
39. Chen1, X liu2, S-L wan3 and C-Y Qin (2010): The Journal of International Medical Research; 38: 498 – 510 [first published online as 38(2) 6.
40. Trevisani F, D'Intino PE, Morselli-Labate AM, et al (2001): Serum alpha-fetoprotein for diagnosis of hepatocellular carcinoma in patients with chronic liver disease: influence of HBsAg and anti-HCV status. JHepatol 2001;34:570–5.
41. Gambarin-Gelwan M, Wolf DC, Shapiro R, Schwartz ME, Min AD (2000): Sensitivity of commonly available screening tests in detecting hepatocellular carcinoma in cirrhotic patients undergoing liver transplantation. Am J Gastroenterol 2000;95:1535–8.
42. Cedrone A, Covino M, Caturelli E, et al (2000): Utility of alpha-fetoprotein (AFP) in the screening of patients with virus-related chronic liver disease: does different viral etiology influence AFP levels in HCC.A study in 350 western patients. Hepatogastroenterology 2000;47:1654–8.

7/31/2017