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ORIGINAL ARTICLE

Serum Angiopoietin-like Protein 3 Could Serve as a Promising Screening Biomarker for Patients with Hepatocellular Carcinoma Before and After Locoregional Therapy

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ABSTRACT

Background: Angiopoietin-like proteins (ANGPTL) proteins play crucial functions in inflammation, lipid metabolism, hematopoietic stem cell activity, as well as cancer cell invasion. This research aimed to evaluate the levels of serum ANGPTL3 in hepatocellular carcinoma (HCC) patients before and after locoregional therapy intervention and to explore if their levels can be utilized in the follow-up of these patients following treatment.

Methods: a case-control study included 50 individuals divided into two groups; the Control group included 25 patients who had chronic liver disease secondary to hepatitis B or C without HCC. The case group included 25 naive HCC patients who were indicated to locoregional therapy (LRT) according to Barcelona Clinic Liver Cancer (BCLC) staging (stage A & B).Serum angiopoietin-like protein 3 level was measured using immunoassay ELISA kit and re-evaluated one month later following intervention.

Results: The HCC group had significantly higher levels of ANGPTL-3 than cirrhotic patients without HCC with a P value <0.001. The best cutoff of AGPTL-3 in the diagnosis of HCC was ≥8.33 ng/ml (p<0.001). the best cutoff of ANGPTL-3 after treatment for prediction of non-viable tumor was 13.91 ng/ml (p<0.001). ANGPTL-3 level >13.19 ng/ml significantly and independently increased the risk of viable/equivocal tumor's response to LRT by 109.6 folds with a p-value of 0.002.

Conclusions: Serum ANGPTL-3 level could serve as a new biomarker to screen for HCC among patients with cirrhosis and could be a possible follow-up marker in the assessment of interventional therapeutic response among HCC patients.

Keywords: Angiopoietin Like Protein 3; Hepatocellular Carcinoma; Locoregional Therapy

INTRODUCTION

Hepatocellular carcinoma (HCC) ranks third among cancers in terms of mortality rates and sixth in terms of frequency of primary malignancies globally [1].Among Egyptian men, HCC ranks first, while among Egyptian women, it ranks second most prevalent cancer. Furthermore, among malignancies in Egypt, it is one of the most common causes of mortality [2].Locoregional therapy (LRT) is one strategy to reduce waitlist dropout caused by tumor growth. The

consensus statement for liver transplantation (LT) for HCC recommends LRT if the anticipated waiting period for an organ to become available is greater than 6 months. While waiting for a transplant, however, the majority of patients undergo LRT as a downstaging step to avoid tumor progression. The most prevalent type of locoregional therapy is transarterial chemoembolization (TACE), although ablation and transarterial radioembolization (TARE) have also been used. Tumor size/number, location, liver function, and individual center experience all play a role in deciding which LRT to utilize [3].Not only is LRT employed as a strategy to reduce waitlist reduction during LT, The Barcelona-Clinic Liver Cancer classification classifies HCC as intermediate-stage, and TACE is the gold standard for patients with this stage. It improves median survival from 16 to 20 months and achieves a partial response in 15%-55% of patients. Another effective method for treating small HCCs that cannot be surgically removed is radiofrequency thermal ablation (RFA), which is gaining popularity [4].A family of proteins with structural similarities to the angiopoietin (ANG) family is known as angiopoietin-like proteins (ANGPTLs) recently discovered. Only eight ANGPTLs have been identified. One interesting aspect to consider is that ANGPTL proteins exhibit multibiological properties, including inflammation, hematopoietic stem cell activity, cancer cell invasion, lipid metabolism, and Angiopoietin-like protein 3 (ANGPTL3), which can stimulate neovascularization and contributes to cancer growth and invasion, similar to vascular endothelial growth factor-a [5].Few studies suggested using ANGPTL3 as a noninvasive diagnostic tool in patients with HCC because of the increased levels of this protein in their serum, which may serve as a predictor for cancer. Considering this gap, our study aimed to evaluate the levels of serum ANGPTL3 in hepatocellular carcinoma patients before and after therapy intervention and to explore if their levels can be utilized in the follow-up of these patients following LRT at Zagazig University Hospitals.

METHODS

Between May 2023 to March 2024, we performed this case-control study on 50 individuals recruited from the Gastroenterology and Hepatology Unit of the Internal Medicine Department, the Interventional Radiology Unit, the specialized liver center in collaboration with the Clinical Pathology department, Zagazig University Hospitals.Written informed consent was obtained from all participants after explaining the procedure and medical research. The research was conducted under the World Medical Association's Code of Ethics (Helsinki Declaration) for human research. This study was carried out after the approval of the Institutional Review Board (IRB) (#10381).

Inclusion criteria were, individuals aged 18 or older with chronic liver disease secondary to hepatitis B or C with and without HCC. HCC naive patients were selected if they were fit and indicated to LRT according to BCLC staging (stages A & B) [6].We excluded patients who had history of any other kind of cancer, whether solid or humoral aside from HCC, those who had organ transplants, individuals who had alcoholism, non-alcoholic fatty liver disease, hepatitis caused by drugs, and other types of viral hepatitis, patients with other diseases that affect serum angiopoietin-like protein 3 levels as coronary artery disease (CAD), end-stage renal disease on maintenance hemodialysis, systemic sclerosis, and hyperlipidemia, patients with contraindication for LRT as patient fit for resection, Liver transplantation, advanced stages (stage C & D), or unfit patients.

Patients were allocated into two groups; the control group included 25 patients who had chronic liver disease secondary to hepatitis B or C without HCC (13 males and 12 females) and the case group included 25 patients with HCC who were fit and indicated to LRT (22 males and 3 females).

All included patients were subjected to full history taking and thorough clinical

examination noting signs of decompensated liver.

Laboratory investigations: Included complete blood count by an automated cell counter (XN-330-sys-Japan), Liver function tests, and Kidney function tests using Roche Cobas 6000 autoanalyzer, HBsAg, HCV Ab, HIV, and Serum α-fetoprotein (AFP) measured by electro chem. Illumine scence on e602 module of Roche Cobas 8000 auto analyzer.

Serum angiopoietin-like protein 3(ANGPTL-3) levels were measured using immunoassay ELISA kit in all patients and were re-evaluated one month later following intervention in HCC patients using DEVELOP Human Angiopoietin Like Protein 3 (ANGPTL3) ELISA Kit, Catalog No: DLR-ANGPTL3-Hu (Wuxi Donglin Sci & Tech Development Co.). The normal Reference range of ANGPTL3 was 5.15 ± 2.71 ng/ml for the healthy population.

Child–Turcotte–Pugh score was assessed according to serum albumin, serum bilirubin, degree of ascites, prothrombin time, as well as the degree of hepatic encephalopathy (A = 5–6, B= 7–9, and C= 10–15). Model of end-stage liver disease (MELD) score was also calculated [7].

Radiological investigations:Abdominal ultrasound as well as triphasic contrast-enhanced computed tomography scans were performed of the abdomen for confirmation of HCC. In the early arterial phase, hypervascularization is marked by contrast enhancement; however, in the late venous phase, this enhancement quickly diminishes. Triphasic CT was repeated one month following LRT to confirm successful ablation. Liver Imaging Reporting and Data System (LI-RADS) was used for characteristics of focal lesions before and after LRT.

LI-RADS categories before LRT suggesting HCC were either LR-4 (probably HCC), non-rim arterial phase hyperenhancement without washout in delayed phase in cirrhotic liver, or LR-5 (definitely HCC): non-rim arterial phase hyperenhancement with washout in delayed phase in the cirrhotic liver [4].

LI-RADS categories after LRT (LR-TR) were classified as LR-TR nonviable which described treated lesionswith no perceived enhancement or demonstrating only expected posttreatment enhancement patterns, treated lesions with APHE (nodular, masslike, or thick irregular sections), washout appearance, or enhancement comparable to pretreatment tumor) were considered LR-TR viable, and It is not possible to define treated lesions as viable or nonviable due to overlapping enhancement features in LR-TR equivocal, which reveals unusual enhancement patterns, in the absence of technological or patient-related limitations [4].

Interventional treatment used for patients with HCC included Radiofrequency ablation (RFA), TACE, and Alcohol ablation.

***Follow-up and outcome*:**Patients with HCC were followed one month following LRT by laboratory investigations including CBC, LFT, KFT, alpha-fetoprotein, and ANGPTL-3 as well as radiological investigation by triphasic CT for assessment of response to LRT according to LI-RADS categorization (LR-TR).

STATISTICAL ANALYSIS

Statistic Package for the Social Sciences, version 26 (SPSS Inc., Chicago, IL, USA), was used to conduct the data analysis. To ensure that the data followed a normal distribution, the Shapiro Walk test was employed. The results of the qualitative analysis were shown using relative percentages and frequency counts. The difference between the qualitative variables was calculated using the chi-square test ($\chi 2$), Monte Carlo, and Fisher exact tests. For variables that were not parametric, we utilized a Mann-Whitney test, and for those that were, we employed an independent T-test. The quantitative data was compared between the two groups using the Kruskal Wallis test (for data that does not follow a normal distribution) and the one-way ANOVA test (for data that follows a normal distribution). To identify the difference between each pair of groups, we utilized a Bonferroni post hoc test and a pairwise comparison where the difference was statistically significant. To make an accurate diagnosis, the ROC curve was employed to ascertain the optimal cutoff

value for a specific quantitative parameter. The Pearson correlation coefficient was utilized to evaluate the direction and intensity of the relationship between two continuous variables. P<0.05 was established as the level of statistical significance.

RESULTS

Table (1) shows a statistically significant difference between the studied groups as regards gender, presence, and grades of ascites, Child-Pugh's score and class, and MELD score. a larger percentage of studied patients had right lobe affection 80%, 60% had a single lesion, 96% had no portal vein thrombosis (only one patient had small portal vein thrombus), 56% had lesions size < 3cm, 72% were LI-RADS 5 while 28% were LI-RADS 4, 84% were BCLC stage A.

The studied groups differed significantly as regards hemoglobin (p<0.001), albumin (p<0.001), platelet count (p=0. 007), and INR (p<0.001). HCC group had significantly higher levels of ANGPTL-3 than cirrhotic patients without HCC with a P value <0.001, with no statistically significant relation between baseline ANGPTL-3 and Child-Pugh's class either in the case or control group (Table 2).

ROC curve analysis shows that the best cutoff of AGPTL-3 in the diagnosis of HCC was ≥8.33 ng/ml with an area under curve 0.984, specificity 92%, sensitivity 96%, negative predictive value 95.8%, positive predictive value 92.3%, with an overall accuracy of 94% (p<0.001). the best cutoff of ANGPTL-3 after treatment for prediction of non-viable tumor was 13.91 ng/ml with area under curve 0.929, specificity 92.9%, sensitivity 90.9%, negative predictive value 92.9%, positive predictive value 90.9%, with overall accuracy of 92% (p<0.001) (Table 3, and Figure 1 A-B).

Significant higher values of ANGPTL-3 in larger HCCs compared to smaller ones were found before treatment (p value=0.027). On doing a post hoc test, the difference was significant between patients with masses <3 cm and those >5cm, however following treatment no significant difference was found. In addition, there was a significant relation between ANGPTL-3 level and the response to LRT with significantly lower levels in patients who achieved LIRADS non-viable with p-value <0.001. On doing a post hoc test, patients with non-viable tumors differ significantly from all other groups (Table 4, Figure 1).ANGPTL-3 levels differed significantly between patients with different responses to LRT with a significantly lower value in patients who achieved LI-RADS non-viable compared to patients with LI-RADS equivocal or viable. In addition, patients with LI-RADS non-viable and equivocal had a significant drop of ANGPTL-3 level following LRT compared to its level before treatment with p-value <0.001 in both groups, however, patients who had viable tumors didn't have a significant drop of ANGPTL-3 level (Table 5, figure 1 C).Multivariate analysis of factors associated with after-treatment viable/equivocal tumor response shows that only ANGPTL-3 level >13.19 ng/ml significantly and independently increased the risk of viable/equivocal tumor's response to LRT by 109.6 folds with p value 0.002 (Table 6).

Statistically significant negative correlations were found between baseline ANGPTL-3 and age of HCC patients (p value=0.016) while, no significant correlation was found with other parameters (**Table S1**).

Table (1): Comparison between the studied groups regarding demographic clinical data, and Disease-specific data among HCC patients

	Case group	Control group	t	p
	Mean ± SD	**Mean ± SD**		
Age (year)	64.76 ± **5.46**	61.84 ± **8.58**	1.436	0.157
	N=25 (%)	**N=25 (%)**	χ^2	**p**
Gender: Female Male	 3 (12%) 22 (88%)	 12 (48%) 13 (52%)	7.714	**0.005***
Comorbidities NAD Diabetes Hypertension Ischemic stroke Cardiac Rheumatoid	 9 (36%) 14 (56%) 11 (44%) 0 (0%) 2 (8%) 1 (4%)	 8 (32%) 15 (60%) 10 (40%) 1 (4%) 0 (0%) 0 (0%)	 0.089 0.082 0.082 Fisher Fisher Fisher	 0.765 0.774 0.774 >0.999 0.49 >0.999
Virology HCV HBV HCV, HBV	 24 (96%) 0 (0%) 1 (4%)	 23 (92%) 2 (8%) 0 (0%)	 MC	 0.62
Ascites: No Mild Moderate Marked	 22 (88%) 2 (8%) 1 (4%) 0 (0%)	 9 (36%) 2 (8%) 3 (12%) 11 (44%)	 17.091¥	 **<0.001****
Child's score: A B C Mean ± SD	 23 (92%) 2 (8%) 0 (0%) 5.32 ± 0.75	 7 (28%) 12 (48%) 6 (24%) 8.36 ± 2.25	 19.376¥ -6.404	 **<0.001**** **<0.001****
	Median (IQR)	**Median (IQR)**	Z	**p**
MELD	8(8 – 10)	12(11 – 19)	-4.206	**<0.001****
Disease-specific data among HCC patients				
	N=25	(%)		p
location: Right Left Both	 20 2 3	 80% 8% 12%		 <0.001**
Number of lesions Single Two Multiple	 15 6 **4**	 60% 24% 16%		 0.424
Size of lesion <3 cm 3 – 5 cm >5 cm	 **14** **2** **9**	 56% 8% 36%		 <0.001**
PV patency: Patent Small thrombosis	 24 1	 96% 4%		 <0.001**
BCLC A B	 21 4	 84% 16%		 0.001**
LIRADS 4 5	 7 18	 28% 72%		 0.043*
Therapy: TACE RF Alcohol ablation	 21 3 1	 84% 12% 4%		 <0.001**
	Median (IQR)	**Range**		
AFP	10.1(3.75 – 65.1)	1.08 - 4293		<0.001**

T independent sample t-test Z Mann Whitney test χ^2Chi square test ¥Chi square for trend test **p≤0.001 is statistically highly significant MC Monte Carlo test NAD: no abnormality detected MELD: Model of end-stage liver disease

Table (2): Comparison between the studied groups regarding laboratory data, and the relation between baseline ANGPTL-3 and Child-Pugh's class among HCC and control groups

	Case group	Control group	t	P
	Mean ± SD	**Mean ± SD**		
Hemoglobin (g/dl)	12.54 ± 1.49	10.06 ± 2.81	3.901	**<0.001****
Albumin (g/dl)	3.97 ± 0.46	2.82 ± 0.76	6.416	**<0.001****
INR	1.16 ± 0.13	1.43 ± 0.3	-4.24	**<0.001****
	Median (IQR)	**Median (IQR)**	Z	**P**
TLC (10^3/mm^3)	5.6(4.15 – 7.7)	4(2.65 – 8.5)	-1.058	0.29
Platelet	122(92 – 227.5)	80(56 – 133)	-2.678	**0.007***
T. bilirubin (mg/dl)	0.9(0.78 – 1.19)	1.2(0.75 – 1.89)	-1.33	0.183
ALT	24(18.5 – 35)	22(16.5 – 28)	-1.321	0.187
AST	28(23 – 47)	31(22.4 – 44)	-0.087	0.93
Serum creatinine (mg/dl)	1(0.8 – 1.1)	0.88(0.72 – 1.85)	-0.282	0.778
ANGPTL-3 (ng/ml)	18.66 ± 5.09	5.15 ± 2.71	11.716	<0.001**

Baseline ANGPTL-3 and Child-Pugh's class among HCC and control groups						
	Case group	T	p	Control group	F	P
	Mean ± SD			**Mean ± SD**		
CPS:						
A	18.29 ± 5.14			5.97 ± 2.18		
B	22.95 ± 1.39	-1.257	0.221	5.02 ± 3.32	0.524	0.599
C				4.43 ± 1.91		

t independent sample t-test Z Mann Whitney test **p≤0.001 is statistically highly significant *p<0.05 is statistically significant, CPS: Child-Pugh's score

Table (3) : Cutoff of baseline ANGPTL-3 for diagnosis of HCC and non-viable tumor in HCC

Cutoff of baseline ANGPTL-3 for diagnosis of HCC							
Cutoff	AUC	Sensitivity	Specificity	PPV	NPV	Accuracy	P
≥8.33	0.984	96%	92%	92.3%	95.8%	94%	<0.001**
Cutoff of baseline ANGPTL-3 for diagnosis of non-viable tumor in HCC							
Cutoff	AUC	Sensitivity	Specificity	PPV	NPV	Accuracy	P
≤13.91	0.929	90.9%	92.9%	90.9%	92.9%	92%	<0.001**

**p≤0.001 is statistically highly significant AUC area under curve PPV positive predictive value NPV negative predictive value **p≤0.001 is statistically highly significant.

Table (4): Relation between ANGPTL-3 before and after therapy and disease-specific data of HCC patients

	Before	T	p	After	t	p
	Mean ± SD			**Mean ± SD**		
location:						
Right	19.26 ± 4.8			13.72 ± 6.37		
Left	15.86 ± 0.35	0.677	0.518	12.52 ± 5.95	0.262	0.772
Both	16.55 ± 8.59			10.85 ± 8.54		
Number of lesions						
Single	18.81 ± 3.77	0.159	0.877	12.82 ± 4.81	-0.385	0.706
Multiple	18.44 ± 6.85			13.96 ± 8.46		
Size of lesion		F			F	
<3 cm	16.32 ± 5.26 [3]	4.281	**0.027***	12.82 ± 7.83	0.163	0.85
3 – 5 cm	21.81 ± 4.67			15.62 ± 0.99		
>5 cm	21.6 ± 2.89			13.47 ± 5.8		
PV patency:						
Patent	18.72 ± 5.19	-0.256	0.8	13.16 ± 6.48	0.432	0.67
Small thrombosis	17.36			16.02		
BCLC						
A	18.52 ± 5.07	-0.22	0.822	14.17 ± 6.28	1.668	0.109
B	19.19 ± 5.98			8.57 ± 5.21		
LIRADS		T	p			
4	14.26 ± 4.7			10.99 ± 7.21	-1.162	**0.272**
5	18.54 ± 3.84	0.03	0.971	14.17 ± 6.0		
LIRADS(LR-TR)					**F**	**p**
Non-viable				7.79 ± 4.94 [2,3]	17.284	**<0.001****
Equivocal				17.2 ± 1.89		
Viable				18.66 ± 5.5		
Therapy:		F			F	
TACE	18.87 ± 5.13			13.84 ± 6.6		
RF	18.02 ± 6.6	0.155	0.857	10.99 ± 5.33	0.557	0.581
Alcohol ablation	16.11			8.31		

t independent sample t-test F One way ANOVA test **p≤0.001 is statistically highly significant *p<0.05 is statistically significant BCLC: Barcelona clinic liver cancer LIRADS: Liver Imaging Reporting and Data System TACE: Transarterial chemoembolization RF: Radiofrequency

Table(5): Relation between response to locoregional therapy according to LI-RADS system and ANGPTL-3 before and after therapy among HCC group

ANGPTL-3 (ng/ml)	Viable (n=5)	Equivocal(n=9)	Non-viable (n=11)	F	p
	Mean ± SD	**Mean ± SD**	**Mean ± SD**		
Before	16.51 ± 5.5	19.93 ± 2.27	18.6 ± 6.5	0.713	0.501
After	18.66 ± 5.5	17.2 ± 1.89	7.79 ± 4.94	17.284	0.001**
Bonferroni	P_1>0.999	**P_2<0.001****	**P_3<0.001****		
p¥	0.161	**0.025***	**<0.001****		
	Median (IQR)	**Median (IQR)**	**Median (IQR)**	**KW**	**p**
% change	7.61(3.26 – 23.55)	-8.38(-17.77, -4.29)	-61.33(-67.26, -49.67)	20.311	<0.001**
Pairwise	P_1 0.083	**P_2 0.003***	**P_3<0.001****		

F One way ANOVA test KW Kruskal Wallis test p for paired sample t-test *p<0.05 is statistically significant **p≤0.001 is statistically highly significant

Table (6) : Multivariate analysis of factors associated with after-treatment viable/equivocal tumor response:

	β	p	AOR	95% C.I.	
				Lower	Upper
Size <3 cm		0.997			
Size 3 – 5 cm	-.122	0.936	0.885	0.045	17.335
Size >5 cm	18.732	0.999	136547117.3	0.000	.
ANGPTL.3 (>13.19)	-0.697	0.002*	109.6	0.001	1995.124

AOR adjusted odds ratio CI Confidence interval

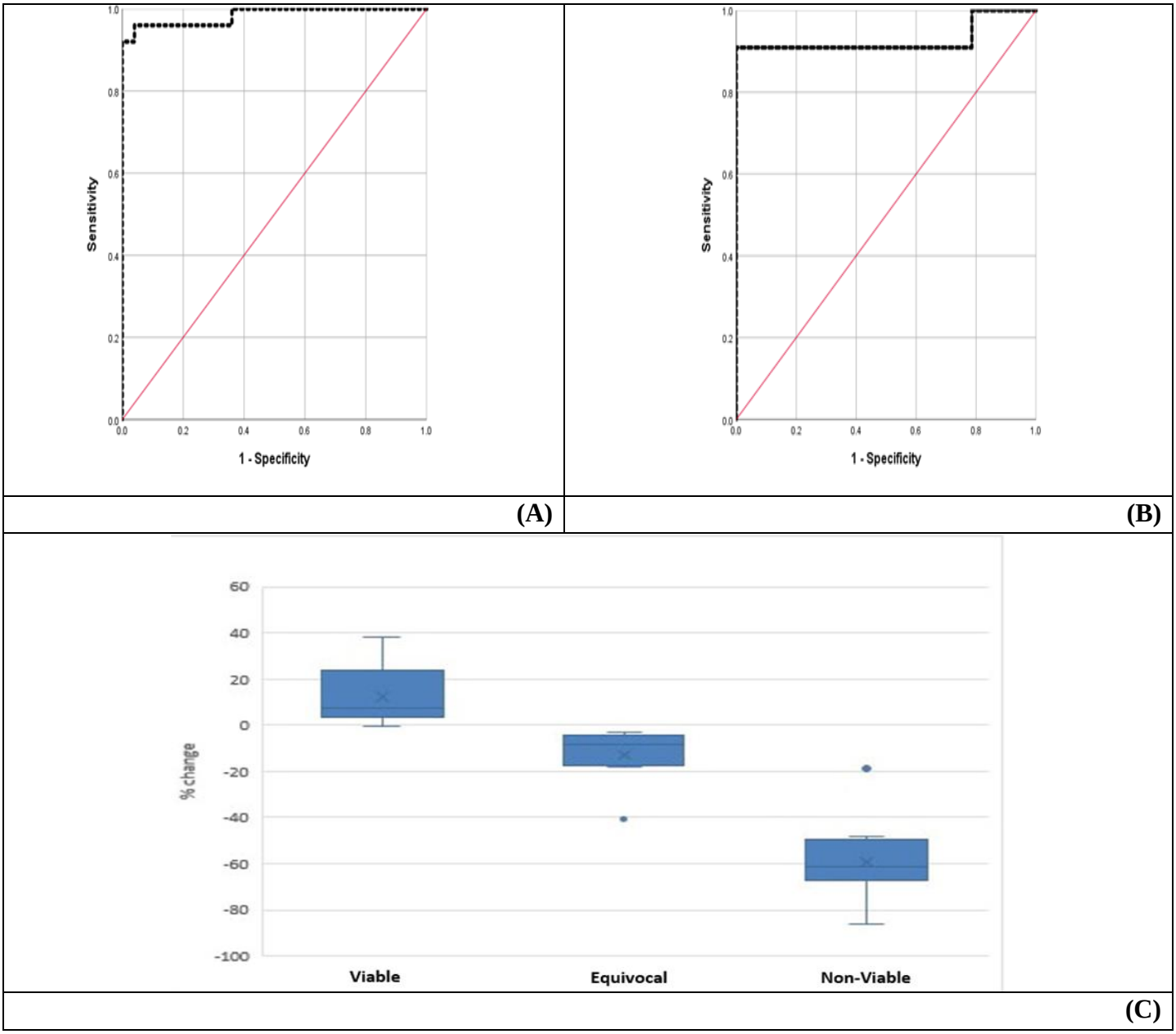

Figure 1: (A): ROC curve showing the performance of baseline ANGPTL-3 for diagnosis of HCC, (B): ROC curve showing the performance of ANGPTL-3 following therapy in prediction of non-viable tumor, (C): Boxplot showing % of drop in ANGPTL-3 level in each treatment's response group

DISCUSSION

In the present study, most HCC patients were males (88%) which was in accordance Bosch et al. [8] that confirmed that the occurrence of HCC is two to four times higher in males compared to females, and it mostly affects men [9].In the early stages of hepatocellular carcinoma, androgen/androgen receptor (AR) speeds up cell proliferation and viral infection; in contrast, estrogen/estrogen receptor (ER) induces cell death and immunological responses. [10]

Of all the liver cancer staging systems used in Western countries, the one first offered in 1999 by the Barcelona Clinic is by far the most popular. 84% of HCC patients in the current study were BCLC stage A and 16% stage B which was compatible with guidelines that recommend ablation or TACE as a treatment option for these stages in patients unfit or refuse resection or transplantation with good physical status [11].We found that the HCC group experienced significantly more elevated levels of serum ANGPTL-3 than cirrhotic patients without HCC with a P value <0.001. Similar to our results in El-Shal et al. [5] study that compared 120 patients with liver cirrhosis to 80 patients with HCC, the researchers used enzyme-linked immunosorbent assays to measure serum levels of ANGPTL3 and ANGPTL4. The HCC cases had significantly higher levels of circulating ANGPTL3 and ANGPTL4 expression compared to the chronic hepatitis patients and controls. Additionally, patients with HCC and chronic hepatitis had significantly higher serum ANGPTL3 and ANGPTL4 values than the control group. The serum ANGPTL3 level (ng/ml) was 242.35 ± 64.50 versus 267.46 ± 87.73 in patients without HCC versus HCC ($P < 0.05$).However, in Valiakou et al. [6] study, statistical analysis revealed that ANGPTL-3 serum levels varied significantly across the different phases of HCV-induced liver illness in 141 samples taken from individuals with different degrees of hepatic stiffness and HCV-induced HCC (p-value = 0.002), with the acute infection group demonstrated the most elevated concentration over the HCC group. This might be because they distinguished between a normal liver, advanced fibrosis, and cirrhosis using the non-invasive Fibroscan® transient elastography. In our research, a cutoff value for ANGPL-3 ≥8.33 ng/ml was beneficial for discriminating HCC patients from the non-HCC group with moral significance making this marker highly helpful in the diagnosis of HCC, however in El-Shal et al. [5] study, they demonstrated that serum ANGPTL3 as well as ANGPTL4 levels failed in distinguishing HCC patients from chronic hepatitis patients ($P = 0.12$). The results showed that ANGPTL3 and ANGPTL 4 expression were better indicators of HCC diagnosis compared to chronic hepatitis ($P < 0.001$). They concluded that ANGPTL3 and ANGPTL4 expression and serum levels show promising roles for the diagnosis of HCC; Specifically, their expression could help differentiate HCC from those with chronic hepatitis.

This difference may be attributed to the varied numbers, and characteristics of patients in our work compared to El-Shal et al. [5] study and distinctive methods of measurement, however, current research paved the road for upcoming investigations needed for more assessment of angiopoietin-like proteins in HCC.In addition, in El-Shal et al. [5] study, a higher risk of HCC was found to be related to elevated levels of ANGPTL3 expression, serum ANGPTL 3, alpha-fetoprotein (AFP), as well as Child-Pugh grade according to multiple-stepwise linear regression analysis. The sole factors that might be used to predict HCC, according to logistic regression analysis, were ANGPTL 3 expression and AFP levels (odds ratio (OR) = 8.9 and 8.6, respectively, $P = 0.003$).Our results observed

no significant relation or correlation of ANGPTL3 to Child's class and score in the HCC group that was not following the El-Shal et al. [5] study which found a significant correlation with Child-Pugh grade with p= 0.01, this also attributed to diverse populations in both studies as they included patients with progressive stages (Child's C) which were excluded in our work because they are contraindicated for any treatment interventions. For similar reasons they found strong direct relationships between ANGPTL3 levels and various clinicopathological characteristics of hepatocellular carcinoma (HCC), such as portal vein thrombosis, lymph node metastasis, tumor lesion count, clinical stage, tumor location, and distant metastasis [5], our study didn't include patients with progressive cancer stages as they are unfit for locoregional therapy, in addition, most of our patients had a single lesion.We agreed with El-Shal et al. [5] study that ANGPTL3 levels were significantly more elevated in larger-sized tumors compared with small tumors before treatment (p = 0.02) making this marker a good prognostic one. Possible explanations include an uptick in carcinogenic cells and ANGPTL3's role in cancer cell development and metastasis.More recent national guidelines for early HCC screening have included novel serum markers such as AFP-L3 and Des-carboxy-prothrombin (DCP) [12]. In addition, there is insufficient evidence for the use of composite risk scores for disease monitoring; however, a composite score that includes imaging and selective serum markers may be able to improve the sensitivity and specificity of these markers alone, like the GALAD score in the early disease setting [13].Despite some evidence suggesting these serum markers can help with prognosis in patients after LRT, they have not been extensively used outside of research [14].For this reason, we tried to investigate the role of ANGPTL3 as a follow-up marker following LRT and to the best of our knowledge this was the first study to explore that. In our study, patients with HCC were re-evaluated one month after their interventional treatment by measuring serum ANGPTL3 levels using an immunoassay ELISA kit and triphasic CT.

The present study revealed a significant relation between ANGPTL3 levels and response to LRT with significantly more reduced values in patients who achieved complete response on CT imaging as shown to be LR-TR nonviable tumors compared with a viable or equivocal response ($p < 0.001$).

In addition, patients with post-treatment non-viable and equivocal response tumors showed a significant drop in ANGPTL3 levels compared to their pretreatment levels ($p < 0.001$ for both), however, patients with viable tumors didn't show a significant change in ANGPTL3 levels.

Could ANGPTL3 be used as a follow-up marker of tumor response to LRT soon? The answer needs more research on a large scale of patients to confirm our results.To strengthen our results, we tried exploring the best cutoff point of ANGPTL3 for the diagnosis of non-viable response to LRT and we found that a post-treatment cutoff value $\leq$ 13.9 had 90.9% sensitivity and 92.9% specificity with p-value <0.001. Furthermore, only post-treatment levels >13.19 were independently and significantly increased the risk of viable/equivocal response by 109.6 folds with a p-value of 0.002.

This study has some points of strength, first, we included patients from 2 centers, and we compared chronic liver disease secondary to hepatitis B or C without HCC with those who had HCC, additionally, serum Angiopoietin Like Protein 3 was measured in two sets at baseline and after one month of intervention.

LIMITATIONS

There may be some bias in evaluating

ANGPTL-3 levels in the follow-up of HCC patients receiving interventional therapy due to the small sample size of the current investigation. We didn't determine ANGPTL-3 gene expression levels and used only their serum level, in addition, we didn't include patients with progressive BCLC stage to evaluate the prognostic role of ANGPL-3 and its correlation to clinicopathological features of HCC.

CONCLUSIONS

Serum ANGPTL-3 level could be a new biomarker to screen for HCC among cirrhotic patients and could be a possible follow-up marker in the assessment of LRT response among HCC patients.

Further large-scale and longer follow-up studies on varied patients' characteristics are needed to confirm the utility of ANGPL-3 as a diagnostic, prognostic, and follow-up marker following various treatment approaches of HCC patients. In addition, more studies to investigate the efficacy of drugs targeting ANGPTL3 as inhibitors or antagonists to serve as therapeutic options for HCC are needed.

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Figure Legends

Figure 1: ROC curves showing (A): the performance of baseline ANGPTL-3 for diagnosis of HCC, (B): the performance of ANGPTL-3 following therapy in the prediction of non-viable tumor. (C): Boxplot showing % of drop in ANGPTL-3 level in each treatment's response group

Supplementary Table (S1) : Correlation between baseline ANGLPT-3 and studied parameter among HCC patients

	r	p
Age (year)	**-0.477**	**0.016***
CPS	0.143	0.494
MELD	-0.27	0.193
Hemoglobin (g/dl)	0.087	0.678
TLC (10^3/mm^3)	-0.075	0.722
Platelet	-0.08	0.704
Albumin (g/dl)	-0.099	0.638
INR	-0.166	0.429
T. bilirubin (mg/dl)	0.021	0.922
ALT	-0.238	0.252
AST	-0.319	0.121
Serum creatinine (mg/dl)	-0.33	0.107
AFP	-0.069	0.745

r Pearson correlation coefficient *p<0.05 is statistically significant

MELD: Model of end-stage liver disease, CPS: Child-Pugh's score, TLC: Total Leucocyte Count, INR: International Normalized Ratio, ALT: Alanine Transaminase, AST: Aspartate Aminotransferase. AFP: Alpha Fetoprotein

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