Angiopoietin-2 as A Diagnostic Biomarker for Liver Cirrhosis and Hepatocellular Carcinoma

Original Article

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ABSTRACT

Background: Cancers like hepatocellular carcinoma are hyper-vascular, and Angiogenesis has a big role in their progression. One of the angiogenic factors that may be useful in diagnosing HCC is angiopoietin-2. In our study, our goal was to estimate the serum Angiopoitin-2's diagnostic utility in identifying liver cirrhosis and HCC.

Subjects and Methods: This case-control research had been carried out at the Kobri Elkoba Military Medical Complex on 117 Subjects were separated in three groups: 39 patients with hepatocellular cancer and liver cirrhosis caused by HCV (HCC group), 39 patients with liver cirrhosis who were age- and sex-matched (cirrhosis group), and 39 healthy volunteers (control group). All participants were subjected to standard Laboratory investigations including serum Angiopoiten-2 measurements, together with abdominal ultrasonography, and Triphasic abdominal CT scan for the two patients' groups.

Results: Serum Angiopoiten-2 is negatively correlated with Albumin and positively with INR, and total Bilirubin. Angiopoietin is significantly related to CT detected portal vein thrombosis in HCC patients. At a cutoff of 145 pg/mL, Ang-2 may be able to discriminate between liver cirrhosis and healthy controls with a sensitivity of 59% and specificity of 64.1%, and with 69.2% and a specificity of 64.1% distinguish between HCC and healthy controls. The sensitivity and specificity of Ang2 to distinguish between HCC and liver cirrhosis were 51.3% and 71.8%, respectively, at a cut-off of 165 pg/mL.

Conclusion: There is a probability that angiopoietin-2 could be adopted as a biomarker for HCC and liver cirrhosis. A bigger sample size and more time are needed for follow-up after HCC interventional therapy in order to confirm the findings and characterize the prognostic role of the angiopoietin Tie2 system.

Key Words: Angiopoietin 2; hepatocellular carcinoma.

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BACKGROUND

In hepatocellular tumor, angiogenesis is crucial to the development of the tumor and the spread of its metastases[1].

In small solid tumors (1 to 2 mm in diameter) it is usually not necessary to create new vessels, but in the case of enlargement it is. This angiogenesis process is under control by an equilibrium of pro- and anti-angiogenic factors[2].

Several substances, including tyrosine kinase with immunoglobulin (Ig) homology domain 2 (TIE2), vascular endothelial growth factor (VEGF), and platelet-derived growth factor (PDGF), are implicated in angiogenic signaling[3]. Angiostatin, Endostatin, and Thrombospondin 1 are examples of endogenous angiogenic suppressors[4].

The pro-angiogenic consequence that supports tumor progression is known as the "angiogenic shift," which typically happens when the balance between pro- and anti-angiogenic elements[5].

Angiopoietins (Ang) include Ang1, Ang2, and Ang3/4. Ang2 is primarily secreted by tumor cells and performs as a Tie2 antagonist. Through modulating the interaction between vascular endothelial and mural cells, the Ang2-Tie2 pathway promotes vascular remodeling[6]. Hypervascular HCCs were found to contain a higher proportion of Ang-2 than hypovascular HCCs. The Ang2-Tie2 signaling pathway may represent a crucial role in the progression and neovascularization of hepatocellular tumor[2].

This study's objective was to assess angiopoietin-2's ability to act as a non- invasive diagnostic marker for liver cirrhosis and hepatocellular carcinoma.

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Subjects and Methods

Research design and setting

It is a case control study executed through August 2021 to June 2022 at the Kobry El Koba Military Medical Complex in Cairo, Egypt.

Participants

117 conveniently chosen adult patients over the age of 18 were involved in the study and separated in three groups. Group A included 39 patients with a confirmed diagnosis of hepatocellular tumor according to the unique appearance of a typical pattern of enhancement on triphasic abdominal CT scan, Group B including 39 age and sex matched post HCV infection liver cirrhosis patients without any hepatic focal lesions excluded by triphasic abdominal CT scan and laboratory investigations while Group C included 39 age- and sex-matched healthy volunteers. Study participants were not included if they had poor renal function, extrahepatic malignancies, HBV infection, or HIV infection. Each individual supplied their informed consent before being included in the research.

Each participant underwent a thorough history taking, a thorough physical examination to look for signs of chronic liver disease, Laboratory investigations such as the complete blood count, blood urea nitrogen, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, bilirubin(total and direct), albumin level , prothrombin time, viral markers like hepatitis C virus antibody, hepatitis B virus surface antigen, in addition to HIV Ab, Alpha fetoprotein level and Angiopiotin2 serum levels were assessed using an ELISA kit (Cat. No. E1221Hu, Human Angiopoietin-2 Quantikine kit) in accordance with the recommendations of the manufacturer. Radiological tests for groups with liver cirrhosis and HCC include abdominal ultrasonography, and triphasic spiral CT abdomen.

Sampling and Sample size

Sample size has been estimated on the basis of assumptions from previous research by Scholz *et al*.[7] on AUC of angiopiotin-2 in discrimination between HCC and cirrhotic patients using Med calc. for sample size on the basis of AUC value of study parameters where: two-sided alpha of .05, power of 0.80 and AUC were 0.77 the minimum required number was 39 for HCC patients, 39 for cirrhotic patients and 39 for healthy controls.

Statistical analysis

The data had been coded, tabulated, and statistically examined using IBM SPSS Statistics 21 (Statistical Package for the Social Sciences). Means and standard deviations (SD) were employed for quantitative data, and frequencies and relative frequencies (percentages) for categorical variables.For quantitative data that were not normally distributed; descriptive statistics were performed using the minimum and maximum of the range, the mean, and the standard deviation. For qualitative data, they were performed using the number and percentage.

When there were two independent groups with normally distributed data, we used the independent t-test; when there were two dependent groups with normally distributed data, we used the paired t-test; and when there were two independent groups with non-normally distributed data, we used the Mann Whitney U test. Inferential analysis for independent variables in qualitative data has traditionally utilized chi-square tests for proportional differences and Fisher's exact tests for variables with tiny, anticipated numbers. To compare quantitative data between various groups, a one-way ANOVA test was utilized. The Kruskal-Wallis test was applied in place of one-way ANOVA for nonparametric data.Post hoc test was used for pairwise comparison after ANOVA test if *P value* is significant.

For numeric normal data, Pearson's correlation was used to perform correlations, and Spearman's rho test was used for numeric non-normal data. The effectiveness of various tests to distinguish between particular groups was evaluated using the ROC curve. The confidence interval was set at 95%, while the allowed margin of error was set at 5%. A *p-value* less than 0.05 was thus considered significant.

Ethical considerations

Armed Forces College of Medicine Ethical Review Committee gave its approval to the study proposal (IRB: 37, meeting: September 25, 2021; serial number: 81). Before being enrolled in the study, all subjects gave written informed consent. based on the specifications of the Revised Helsinki Declaration of Biomedical Ethics, the study was in compliance. Data confidentiality guidelines were rigorously adhered to.

RESULTS

This study included 117 subjects: 39 HCC patients (mean age 65.46±7.69), of which 11 were Child A patients, 9 were Child B patients and 19 were Child C patients, 39 were cirrhotic patients (mean age 55.49±15.12), of which 24 were Child-A, 6 were Child-B and 9 were Child-C and 39 healthy volunteers as controls (mean age 43.72±11.70).In terms of laboratory data, there were statistically significantly lower values for hemoglobin, platelets and serum albumin and higher values for INR, Alanine transaminase, aspartate aminotransferase, total and direct bilirubin when comparing hepatocellular tumor patients and liver cirrhosis patients with healthy controls. In addition, the mean AFP values in the hepatocellular tumor group were statistically significantly higher than in the liver cirrhosis group. (Table 1)

Table 1: Comparison between the studied groups regarding Laboratory investigations

	Group						F**	*P value*
	HCC (N=39)		Liver cirrhosis (N=39)		Normal (N=39)			
	Mean	SD	Mean	SD	Mean	SD		
Hemoglobin (g/dL)	11.08	2.08	12.61	1.97	13.52	0.72	20.39	<0.001
Platelet count (x103/ml)	130.03	64.65	162.4	84.24	264.44	85.13	31.08	<0.001
White cell count (x103/ml)	7	5.6-11.2	6.6	4.6-8.5	5.7	4.9-6.5	12.01	0.002
INR	1.48	0.39	1.23	0.26	1	0	30.58	<0.001
ALT (IU/mL)	77.82	61.85	40.92	36.63	26.18	3.82	15.99	<0.001
AST (IU/mL) *	91	53-152	32	19-48	25	23-27	45.06	<0.001
Alkaline phosphatase (IU/L)	185.33	116.03	77.36	36.67	86.23	33.85	26.35	<0.001
Albumin (g/dL)	2.73	0.61	3.76	1.13	4.29	0.31	41.58	<0.001
Total Bilirubin (mg/dL) *	2.4	1.4-15.8	1.3	0.5-2.4	0.7	0.5-0.8	48.36	<0.001
Direct Bilirubin (mg/dL) *	1.5	0.5-9.7	0.3	0.1-0.7	0.1	0.1-0.2	63.15	<0.001
Creatinine (mg/dL)	1.11	0.32	1.08	0.19	0.96	0.1	5.06	<0.001
AFP (ng/mL) *	10.6	6.4-420	7	6-11			3	0.003

*Median and IQR (Kruskal Wallis test), **One Way ANOVA test, ***Chi square test

The current study demonstrated that median serum level of Angiopoitin-2 was statistically significantly higher in hepatocellular carcinoma group, in comparison to liver cirrhosis and control groups with a *P value* of 0.001. (Table 2)

Table 2: Comparison between serum Angiopoitin-2 (pg/mL) among studied groups

	Group						Kruskal Wallis test	*P value*
	HCC (N=39)		Liver cirrhosis (N=39)		Normal (N=39)			
	Mean	SD	Mean	SD	Mean	SD		
Angiopiotin-2 (pg/mL)	170	130-320	150	130-180	130	130-150	15.1	0.001

Angiopoitin-2 demonstrated a statistically significant positive connection with INR and total bilirubin, with *P values* of 0.031 and 0.041, respectively, when it was compared to various laboratory data. There is a negative association between serum albumin-related and Angiopoitin-2 with a *P value* of 0.02 that is statistically significant. (Table 3).

With a *p value* of 0.03, angiopoitin-2 serum levels demonstrated a significant connection with portal vein thrombosis in CT, although no other CT findings did (Table 4)

The present work also found that there was a non-significant association between serum angiopoitin-2 levels and children's Pugh score or liver cancer stage at the Barcelona Clinic (Table 5, 6)

Table 3: Correlation between Angiopoiten-2 and different laboratory parameters among HCC patients.

	Angiopoitin-2 (pg/mL)	
	Spearman's correlation r_s	Significance *P value*
Hemoglobin (g/dL)	-0.09	0.587
Platelet count (10^3/mL)	-0.008	0.960
White cell count (10^3/mL)	0.288	0.075
INR	0.345	0.031
ALT (IU/mL)	-0.006	0.973
AST (IU/mL)	0.033	0.841
Alkaline phosphatase (IU/L)	-0.056	0.736
Albumin (g/dL)	-0.370	0.020
Total Bilirubin (mg/dL)	0.329	0.041
Direct Bilirubin (mg/dL)	0.199	0.225
Creatinine (mg/dL)	0.168	0.306
AFP (ng/mL)	0.003	0.986

Table 4: Relation between Angiopoiten-2 and CT findings among HCC patients.

		Angiopoitin-2 (pg/mL)		Z*	*P value*
		Median	IQR		
Splenomegaly	No	205	170-310	0.76	0.45
	Yes	160	130-790		
Portal vein	Thrombosed	750	235-1200	2.17	0.03
	Patent	150	130-240		
Ascites	No	160	140-300	0.63	0.53
	Yes	170	130-800		
Lymphadenopathy	No	165	135-555	0.17	0.87
	Yes	170	130-300		
Site	Right lobe	170	130-790	0.59	0.55
	Both	150	150-300		
Size	< 3 cm	170	130-300	0.69	0.49
	> 3 cm	165	140-790		
Number	Solitary	165	130-555	0.02	0.99
	Multiple	170	140-300		

*Mann Whitney U test

Table 5: Relation between Angiopoiten-2 and Child score among HCC patients.

		Angiopoitin-2 (pg/mL)		Kruskal Wallis test	*P value*
		Median	IQR		
Child score	A	150	130-180	2.52	0.28
	B	170	150-180		
	C	240	130-1200		

Table 6: Relation between Angiopoiten-2 and Barcelona clinic liver cancer staging among HCC patients.

		Angiopoitin-2 (pg/mL)		Kruskal Wallis test	*P value*
		Median	IQR		
Barcelona clinic liver cancer staging	Stage 0	130	130-130	9.73	0.05
	Stage A	170	170-180		
	Stage B	150	150-160		
	Stage C	305	300-315		
	Stage D	240	130-1200		

At a cutoff value of 145 pg/mL, the ROC curve suggests that Ang-2 may be able to distinguish between liver cirrhosis and healthy controls with 59% sensitivity and 64.1% specificity, as well as between HCC and healthy controls with 69.2% and 64.1% specificity. (Figures 1, 2)

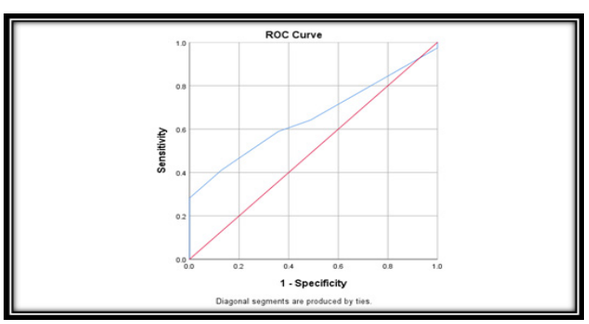

Fig. 1: ROC curve of Angiopoiten-2 for differentiation between liver cirrhosis and healthy controls

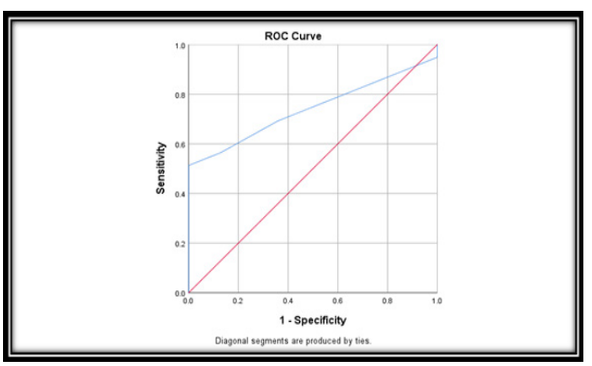

Fig. 2: ROC curve of Angiopoiten-2 for differentiation between HCC and healthy controls

The sensitivity and specificity of Ang2 for differentiating between hepatocellular tumor and liver cirrhosis were 51.3% and 71.8% respectively at a threshold value of 165 pg/mL. (Figure 3)

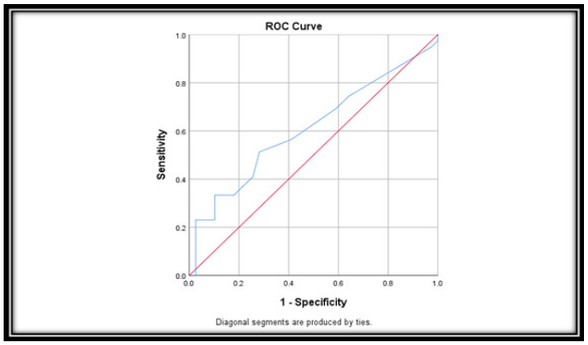

Fig. 3: ROC curve of Angiopoiten-2 for differentiation between HCC and liver cirrhosis

DISCUSSION

With 13,000 new cases worldwide each year, Hepatocellular carcinoma is the second most prevalent cancer that results in death for men and the sixth most common for women[8].

The key element in the development of hepatocellular tumor is liver cirrhosis, but the pathophysiology varies depending on the underlying etiological cause. Important factors contributing to worsening progression and carcinogenesis are angiogenesis, persistent inflammation, and changes in the micro- and macroenvironment of the tumor. HCC development may be influenced by extrinsic risk factors as well as congenital genetic susceptibility[9].

One of the defining characteristics of cancer that encourages tumor growth and metastatic spread is tumor angiogenesis[2].

Tie 1 and Tie2 are tyrosine kinase ligands that have Ig and EGF homology domains, and together they make up the angiopoietin (Ang) family, which includes Ang1, Ang2, and Ang3/4[10]. Tie2 is activated by Ang1, which is primarily produced by smooth muscle cells in the vascular system. In contrast, Ang2 functions as an antagonist for Tie2 and is primarily secreted by tumor cells. The Ang2-Tie2 pathway inhibits communication between endothelial and wall cells, which encourages vascular remodeling. These findings imply that neovascularization and the development of HCC are both significantly influenced by the Ang2-Tie2 signaling pathway[11].

This study's objective was to assess angiopoietin-2's potential as a diagnostic biomarker for liver cirrhosis and hepatocellular carcinoma.

This case-control study was performed at the Kobri Elkoba Military Medical Complex on 39 adult patients > 18 years of age with HCV-cirrhotic liver disease and HCC (HCC group), 39 non-HCC liver cirrhotic patients (cirrhosis group), and 39 healthy volunteers (control) carried out group). Age and gender balance among the three groups was achieved.

Regarding laboratory investigations of the studied groups, Compared to healthy controls, individuals with liver cirrhosis and HCC had statistically significantly decreased levels of hemoglobin which may be due to acute or chronic blood loss, hypersplenism, folate deficiency complicating the course of liver disease or because of the anemia of chronic disease. This finding agrees with Radwan *et al*.[12] who noted that hemoglobin levels in patients with hepatocellular tumor were statistically different lower than in patients with liver cirrhosis and healthy controls, and also Sakisaka *et al*.[13] who showed that despite the high serum erythropoietin levels found in about 25% of HCC patients, the hemoglobin level is not high and most HCC patients are anemic, mostly due to other effects of the cancer.

Also the current study found that Platelet count is significantly much less in both liver cirrhosis and hepatocellular carcinoma as opposed to healthy controls and this could be explained by the effect of portal hypertension which cause sequestration of the platelets in the congested spleen and may be also caused by the decreased levels of thrombopoietin level which is mainly produced by the liver and these results were in agreement with Radwan *et al*.[12] and Zekri *et al*.[14] who revealed that the platelets were significantly lower in HCC and liver disease groups than the control group.

Furthermore, this study found that albumin level is significantly much less in the liver disease and HCC groups in contrast to the control group. Also, we found that the INR is significantly higher in the groups with liver disease and HCC than in the healthy group. This study also found that Alanine transaminase, aspartate aminotransferase, total and direct bilirubin are significantly higher in HCC and liver disease groups than in the control group. The outcomes of this study agreed with those of Rowida *et al*.[16] and Bannaga *et al*.[15] research.

In addition, Zekri *et al*.[14] showed that both patient groups (cirrhosis and HCC groups) had highly significant increases in aspartate aminotransferase, Alanine transaminase, and total bilirubin when compared to controls. The present research also found that the mean level of AFP was statistically significantly higher in the hepatocellular carcinoma (HCC) group in comparison to the liver cirrhosis group, which is consistent with past studies by Zekri *et al*.[14] and Nouh *et al*.[17].

Assessing the importance of serum Ang-2 as a diagnostic biomarker for hepatocellular carcinoma, the present work found that the mean Ang-2 value in the HCC group was 170.00 pg/ml, while in the liver cirrhosis group it was 150 pg/ml and in the liver cirrhosis group was 130 pg/ml in the control group. In addition, there were statistically significantly higher levels of Ang-2 in hepatocellular carcinoma (HCC) patients in comparison to healthy controls with (P *value* < 0.001) and significantly higher levels in cirrhotic patients compared to controls (P *value* = 0.05). However, a non-significant difference (P *value* = 0.27) was found between hepatocellular

carcinoma (HCC) and liver cirrhosis patients. Consistent with the present study, Zekri *et al*.[14] aimed to evaluate the role of serum Ang-2 as a diagnostic tumor marker in hepatocellular carcinoma patients with liver cirrhosis. The study found that the HCC group had the highest mean serum Ang 2 levels in comparison to the cirrhotic and healthy control groups, with a p-value of 0.001. Also, Nouh *et al*.[17] and Zhou *et al*.[18] revealed that hepatocellular carcinoma patients have the highest mean levels of serum Ang-2 compared to control and cirrhotic groups.

In addition, Ao *et al*.[2] explored if Ang2 may be used as a biomarker for hepatocellular cancer diagnosis and prognosis and found that mean Ang2 values in controls, cirrhotic patients, and hepatocellular carcinoma patients were 1.58, 2.33, and 3.53 ng/mL, respectively deceive. In the hepatocellular cancer group of the current study, there were significant positive correlations between Ang-2, INR and total bilirubin, with *P values* of 0.031 and 0.041, respectively. In addition, there was a significant negative correlation with albumin (*P-value* 0.02). This is consistent with Isaac *et al*.[19] who claimed that there are significant negative correlations with serum albumin and significant positive correlations between Ang-2 and INR and total bilirubin. This was also supported by Kronsten *et al*.[20], who realized that in HCC patients, Ang-2 levels had *p values* of 0.001 and 0.04, respectively, for positive and negative correlations with INR and albumin.The present study showed insignificant relation between Ang-2 and ultrasonographic findings in HCC patients. Nevertheless, on performing abdominal triphasic CT, Ang-2 showed a significant relationship with portal vein thrombosis with a *P value* of 0.03.

Ao *et al*.[2] also declared no significant relationship between Ang-2 and sonographic results, However, A strong association between tumor size, portal vein thrombosis, and Ang-2 was shown by Nouh *et al*.[17].

The current study showed insignificant relation between Ang-2 and child score although its levels were higher in Child C than Child B and A patients. In agreement with the current study Scholz *et al*.[7] and Nouh *et al*.[17] declared absence of major relation between serum Ang-2 and Child classification.

Our results, however, differ from those of Ao *et al*.[2] and Kronsten *et al*.[20], who found a positive correlation between Ang-2 levels and Child-Pugh score in hepatocellular carcinoma (HCC) patients (r = 0.38, P = 0.003). Additionally, Pestana *et al*.[21] noticed that hepatocellular carcinoma (HCC) patients with higher Child-Pugh scores had significantly higher mean plasma Ang-2 levels.

The current study found no link between Ang-2 and BCLC. Contrary to what Diaz-Sanchez *et al*.[22] reported, plasma levels of Ang-2 increased significantly from early stage BCLC (stage A) to intermediate BCLC (B) and advanced stage HCC BCLC (stage C/D).

To test the validity of Angiopoiten-2 in differentiation between hepatocellular carcinoma (HCC) and healthy controls, ROC curve analysis was performed, declaring that at Cutoff value of ≥ 145 pg/mL Ang-2 had Sensitivity of 69.2% and Specificity of 64.1%.

Regarding the validity of Ang-2 in differentiation between liver cirrhosis and healthy controls, at a Cutoff value of >145 pg/mL Ang-2 was 59% sensitive and 64.1% specific.

As well, regarding the validity of Ang-2 for differentiation between hepatocellular carcinoma (HCC) and liver cirrhosis, the Best Cut off value >165 pg/mL had Sensitivity of 51.3% and Specificity of 71.8%.

Serum Ang-2 levels were observed to be considerably higher in hepatocellular carcinoma (HCC) patients compared to cirrhotic patients and healthy controls by Zekri *et al*.[14]. The sensitivity and specificity for detecting HCC were 96% and 76%, respectively, with an ideal cut-off of 5360 pg/mL and an accuracy of 90%.

Furthermore, Nouh *et al*.[17] discovered that ROC analysis showed 98% sensitivity and 100% specificity for Ang-2 in the differentiation of hepatocellular carcinoma (HCC) patients and healthy controls at a threshold level of 315.5pg.

The appropriate cut-off value for Ang-2 has been established by ROC analysis, according to Ao *et al*.[2], and it represents 3.5 ng/mL. For the identification of HCC, Ang-2 has sensitivity, specificity, and accuracy values of 50.9, 83.7, and 59.5%, respectively.

LIMITATIONS OF THE STUDY

The requirement for a longer duration follow-up time following various treatment methods in order to identify future tumor growth and the relatively small sample size.

CONCLUSION

Angiopoiten-2 can potentially serve as a biomarker for the recognition of hepatocellular carcinoma and liver cirrhosis, according to the findings of our study. The findings need to be confirmed by additional research utilizing a larger sample size and a longer follow-up period following hepatocellular carcinoma interventional treatment in order to establish the predictive role of the angiopoietin Tie2 system in the progression of liver disease.

ABBREVIATIONS

HCC: Hepatocellular carcinoma (HCC); **ANG-2:** Angiopoietin-2; **Ig:** Immunoglobulin; **EGF:** Epidermal growth factor; **VEGF:** Vascular endothelial growth factor; **PDGF:** Platelet-derived growth factor; **Ang:** Angiopoietin; **ALT:** Alanine aminotransferase; **AST:** Aspartate aminotransferase; **AFP:** Alpha-fetoprotein; **ELISA:**

enzyme-linked immunosorbent assay; **BCLC:** Barcelona Clinic Liver Cancer; **INR:** International normalized ratio.

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AUTHORS' CONTRIBUTIONS

OAA designed the study. AA designed the data collection tool. AI conceptualized the study and performed the data analysis and interpretation. IE carried out data collection and wrote the original draft. All authors revised the article before submission.

CONFLICT OF INTERESTS

There are no conflicts of interest

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