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Vascular endothelial growth factor in relation to the development of hepatocellular carcinoma in hepatitis C virus patients treated by direct-acting antivirals

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Abstract

Background: Hepatocellular carcinoma (HCC) comprises 5.6% of all cancers worldwide representing the sixth most common cancer. It is also the fourth most common cause of cancer-related mortality. Angiogenesis is a main factor in the development of HCC. Vascular endothelial growth factor (VEGF) is considered as the force for physiological and pathological angiogenesis, and overexpression of VEGF is prominent in HCC. We aimed to study the effect of direct-acting antivirals (DAAs) on VEGF considered as the key regulator of angiogenesis in HCC. This cross-sectional study involved fifty patients who were divided into two groups: group I—twenty-five chronic hepatitis C virus (HCV) patients as (cases) subjected to treatment with direct-acting antiviral drugs for 3 months; group II—twenty-five chronic HCV patients developed HCC as (controls). Serum VEGF level was measured in of group I at baseline, at end of treatment, and 3 months after the end of treatment by sofosbuvir 400 mg plus daclatasvir 60 mg for 3 months in the HCV patient group, also VEGF was assessed in group II with HCC.

Results: Serum VEGF was high in both groups, but it was higher in the HCC group with a statistically significant difference ($p < 0.001$), also serum VEGF in the HCV group decreased after 3 months at the end of DAA treatment from 209.5 ± 137.6 to 44.1 (31.8–55.3) mg/ml, and all patients who received DAAs achieved sustained virologic response (SVR).

Conclusion: We found that change in serum VEGF in HCV patients treated with DAAs in this study cannot explain the risk of HCC after treatment by DAAs.

Keywords: Vascular endothelial growth factor A, HCV, DAAs, HCC

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Background

Liver cancer occupies the sixth rank among common cancers and the fourth among the leading causes of cancer-associated mortalities [1]. Hepatocellular carcinoma (HCC) is considered the commonest liver cancer worldwide [2]. Chronic infection with HCV is considered the most common etiology for HCC [3]. The direct-acting antiviral agents (DAAs) recently improve the standards of therapy that achieve complete cure of hepatitis C virus infection, which in turn leads to a decreased risk of developing HCC and its associated high mortality [4].

Although the novel therapies are effective in treatment, they did not manage to reduce the risk of HCC in those with higher degrees of liver cirrhosis to zero percent [5]. Cirrhosis plays an important role in the occurrence of HCC since the majority of HCV patients who develop HCC are cirrhotic, and patients with advanced cirrhosis are highly prone to develop HCC even after achieving sustained virologic response (SVR) [6]. Many studies have shown the effect of DAA regimens on HCC recurrence and development; however, neither of them could confirm this relation [1, 7–9].

AFP has limited sensitivity (41–65%) for the detection hepatocellular carcinoma especially in its early phases. It is not secreted in all cases of HCC, and its serum levels may be within normal ranges in about 40% of patients with HCC. So, detection of serum AFP only is not a reliable indicator in the early phases of HCC [10]. Therefore, the presence of an alternative marker for HCC is important. It was reported that vascular endothelial growth factor (VEGF) is only detected in HCC cells but not in benign liver tissues, and can thus be used as an excellent marker for the diagnosis of early HCC [11].

VEGF signaling is the main regulator of angiogenesis, which is impaired in most solid types of cancer as liver tumors' growth needs high vascularity through new blood vessel formation to suffice its increased metabolic demands [12]. HCV can affect the process of angiogenesis through triggering of hypoxia-inducible factor 1 alpha (HIF-1α) that turns into increased VEGF expression [13]. Also, HCV activates the signal transducer and activator of transcription 3 (STAT3) which stimulates both the transcriptional activity of androgen receptors and VEGF expression [14].

We aimed to study the impact of DAAs on VEGF expression which is the key regulator of angiogenesis in HCC.

Methods

We included fifty adult patients from Ain Shams University Hospitals; they were divided into two groups:

- Group I (case group): twenty-five chronic HCV patients as evidenced by positive HCV Ab (at least 6 months duration) and detectable quantitative HCV-RNA were subjected to the treatment of hepatitis C virus by sofosbuvir 400 mg plus daclatasvir 60 mg for 3 months (HCV HCC-free group) and all patients achieved SVR.
- Group II (control group): twenty-five HCV patients who developed HCC. HCC was confirmed by triphasic abdominal CT with contrast verifying criteria of HCC (HCV related HCC patients).

Any causes of chronic liver disease other than HCV such as hepatitis B virus (HBV) were excluded. We also excluded patients with morbid obesity, dyslipidemia, diabetes mellitus, and patients with any malignancy other than HCC. Also, patients with rheumatoid arthritis, pregnant females, and patients with decompensated liver disease (Child-Pugh C) were excluded from the study.

All patients were subjected to the following:

Full history and clinical examination and the following laboratory tests:

- Complete blood count (CBC).
- Serum creatinine.
- Aspartate aminotransferase (AST), alanine aminotransferase (ALT), and serum albumin.
- PCR quantitative for HCV.
- Serum alpha-fetoprotein level (AFP).
- Serum vascular endothelial growth factor normal range (27–30 ng/ml) was assessed for all patients included in both groups.
  - For group I, VEGF was assessed before starting antiviral treatment (baseline) then at the end of treatment, 1 month after the end of treatment, and 3 months after the end of treatment (SVR).
  - For group II, VEGF was assessed at the beginning of the study.
- Radiological examination: pelviabdominal ultrasound.

Sample size calculation

After reviewing the literatures [15, 16], we used G*POWER statistical programming version 3.1.9.4 (Franz Faul, University at Kiel, Germany) to calculate the sample size per group. Using $a = 0.05$, a power of 90%, an effect size of 0.628, and an allocation ration of 1:1 showed that the minimum required sample size to avoid type II error is 58 patients (29 patients in each group) was needed. Four patients were lost to follow-up from each group. So, there were 25 patients in each group.

Statistical analysis

We used SPSS (Statistical Package for Social Sciences) for Windows version 24 (SPSS Inc., Chicago, IL, USA).

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Numerical data were expressed in terms of mean and standard deviation (mean ± SD) if normally distributed or median and inter-quartile range if not normally distributed. Normality was tested using the Shapiro-Wilk test. Pearson and Spearman correlations were used to test the association between the numerical dependent and independent variables. The Friedman test was used to test the difference between paired numerical data. We conducted a linear regression analysis to test the absolute relations between dependent and independent variables. *P* value less than 0.05 was sufficient enough to show the statistical significance.

Results

This study was conducted on fifty patients. Both groups were age- and sex-matched. They were subdivided into two groups.

- Group I (HCV HCC-free group): twenty-five HCV patients were subjected to the treatment of chronic hepatitis C virus by DAAs.
- Group II (HCV-related HCC group): twenty-five HCV patients who developed HCC.

As regards the demographic data, there were no statistically significant differences in age and sex, although all patients had normal body weight but significantly higher median BMI in the HCC group compared with the other ($p < 0.001$). Concerning laboratory findings, patients in the HCC group had statistically significantly lower levels of hemoglobin and platelets than others in the HCV group ($p < 0.001$, $p < 0.001$, respectively), but serum

creatinine levels were significantly higher in the HCC group than in the HCV group ($p < 0.001$).

Also, AFP was dramatically higher in the HCC group ($p < 0.001$) than in the HCV group. Concerning liver functions, bilirubin levels were significantly higher in HCC group ($p < 0.001$), both groups had similar ALT and AST levels ($p = 0.15$, $p = 0.39$, respectively). However, albumin levels were significantly higher in the HCV group ($p < 0.001$) as shown in Table 1.

HCC patients had significantly higher levels of VEGF compared to HCV patients (488.01 ± 194.5 versus 209.5 ± 137.6, respectively).

For HCV patients, there was a positive correlation between baseline VEGF levels and BMI, bilirubin, AST, PCR, and AFP levels. VEGF was negatively correlated with age, Hb levels, platelet count, albumin level, ALT level, and creatinine levels. However, none of those correlations was statistically significant as shown in Table 2.

For HCC patients, there was a positive correlation with age, bilirubin levels, ALT levels, AST levels, and AFP levels. There was a negative correlation between baseline VEGF levels and BMI of patients, Hb levels, platelets count, Alp levels, and creatinine levels. Only AST levels showed a significant correlation with baseline VEGF in HCC patients ($p < 0.001$) as shown in Table 2.

On follow-up of HCV patients, treatment with DAAs could significantly decrease VEGF values at the end of the treatment period, 1 month after treatment ($p < 0.001$), and even 3 months post-treatment (Table 3).

There was a positive correlation between VEGF values and age of patients, platelets count, bilirubin levels Alp levels, and PCR values. Age of patients and PCR values

Table 1 Comparison between the 2 groups regarding demographic and laboratory findings

	HCV group (*n* = 25)	HCC group (*n* = 25)	*P* value
Gender[#]			
Male	17 (50)	17 (50)	1.00
Female	8 (50)	8 (50)	
Age (years)	58.6 ± 4.4*	59 ± 4.2*	0.74
BMI	20 (19–22)**	24 (22–25)**	< 0.001
Hemoglobin (gm/dl)	13 (11.75–15)**	9.54 ± 1.33*	< 0.001
Platelets (10^3/Ul)	220 (153.5–240)**	89.8 ± 17.76*	< 0.001
Bilirubin (mg/dl)	0.8 (0.7–0.95)**	2.4 (1.9–3.9)**	< 0.001
Albumin (g/dl)	3.82 ± 0.34*	2.99 ± 0.45*	< 0.001
ALT (U/L)	46 (27.5–65) **	41.72 ± 9.66*	0.15
AST (U/L)	45.28 ± 22.4*	40.88 ± 12.09*	0.39
Creatinine (mg/dl)	1 (0.8–1.03) **	1.3 (1–1.75) **	0.001
AFP (ng/ml)	4.5 (3.2–6.65) **	311.4 ± 209.43*	< 0.001
VEGF (ng/ml)	209.5 ± 137.6*	488.01 ± 194.5*	< 0.001

*Data represented in terms of mean ± SD
**Data represented in terms of median (Q1–Q3)
[#]Data represented in terms of frequency (percentage), *n* (%)

Table 2 Correlation between baseline VEGF and laboratory findings in both groups

Variable	HCV group ($n = 25$)		HCC group ($n = 25$)	
	Correlation coefficient	P value	Correlation coefficient	P value
Age (years)	− 0.238[P]	0.251	0.281[P]	0.174
BMI	0.06[S]	0.77	− 0.142[P]	0.5
Hb (gm/dl)	− 0.12[S]	0.56	− 0.344[P]	0.09
Platelets (10^3/Ul)	− 0.14[S]	0.5	− 0.07[P]	0.72
Bilirubin (mg/dl)	0.05[S]	0.83	0.07[P]	0.74
Albumin (g/dl)	− 0.161[P]	0.441	− 0.37[P]	0.07
ALT (U/L)	− 0.02[S]	0.93	0.07[P]	0.713
AST (U/L)	0.086[P]	0.68	0.67[P]	< 0.001
Creatinine (mg/dl)	− 0.215[S]	0.3	− 0.03[P]	0.89
HCV-PCR	0.11[S]	0.6		
AFP (ng/ml)	0.325[S]	0.113	0.02[P]	0.93

[P]Pearson correlation
[S]Spearman correlation

were significant determinants of SVR VEGF values ($p = 0.04$, $p = 0.03$, respectively) as shown in Table 4.

After adjustment of the co-founding factors through linear regression analysis, we found that neither age of patients nor PCR values were significant predictors of the VEGF value 3 months post-treatment by DAAs ($p = 0.064$, $p = 0.219$, respectively) as shown in Table 5.

Discussion

The modern use of direct-acting antiviral drugs (DAAs) in the treatment of HCV has a major advantage in achieving more than 90% SVR despite the degree of liver cirrhosis. This allowed the treatment of many more patients, some of them with more advanced liver function impairment and more susceptibility for HCC [17]. However, the impact of DAA-based treatment on HCC occurrence in patients with cirrhosis is still controversial.

In this study, we found high statistically significant higher VEGF serum level in HCC patients than in HCV patients reflecting the pro-inflammatory and pro-angiogenic state that continued from benign to malignant liver disease and the impact of VEGF in addition to other angiogenic and growth factors on the growth, propagation,

and spread of HCC as previously reported by Villani et al. [16], Debes et al. [18], and Faillaci et al. [19].

In spite of the great advancement in the HCV treatment after DAA introduction and achievement of more than 90% SVR in respective of fibrosis stage, which will improve the HCV natural history of infection, however, several recent reports have raised concerns about DAA treatment because of higher rates of HCC recurrence observed after DAA therapy [16, 20] and even early occurrence of HCC after treatment by DAAs [21].

We addressed in this study the possibility that serum VEGF after successful viral eradication by DAAs may be one of the molecular mechanisms responsible for early occurrence and recurrence of HCC as reported by Villani et al. [16]; however, we found that serum VEGF in our studied patients dropped after 1 month and 3 months after DAA treatment in controversy to the finding of Villani et al. who reported that although DAAs reduce the inflammation, they increase serum VEGF level and they considered this as a rationale for HCC occurrence risk during anti-HCV treatment; however, due to different finding in the present study in our patients, this reflects the different population number and characteristics

Table 3 VEGF through the study period

HCV group	VEGF1	VEGF2	VEGF3
Values (median (Q1–Q3))	173.4 (146.6–199.3)	42.2 (31.3–78.3)	44.1 (31.8–55.3)
P value	< 0.001		
Post hoc			
VEGF1–VEGF2	< 0.001		
VEGF1–VEGF3	< 0.001		
VEGF2–VEGF3	0.09		

VEGF1 measured at the end of treatment, VEGF2 measured one month after the end of treatment, and VEGF3 measured 3 months after the end of treatment

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Table 4 Correlation between 3 months VEGF values and laboratory findings in HCV HCC-free patients

Variables	Correlation coefficient	P value
Age (years)	0.414[P]	0.04*
BMI	− 0.34[S]	0.09
Hemoglobin (g/dl)	− 0.16[S]	0.44
Platelets(10^3/Ul)	0.11[S]	0.59
Bilirubin (mg/dl)	0.003[S]	0.99
Albumin (g/dl)	0.13[P]	0.54
ALT (U/L)	− 0.037[S]	0.86
AST (U/L)	− 0.025[P]	0.9
Creatinine (mg/dl)	− 0.067[S]	0.75
HCV-PCR	0.44[P]	0.03*

[P]Pearson correlation
[S]Spearman correlation
*Statistically significant

included in different studies and reflect the multi-factorial explanation for HCC occurrence after DAA therapy.

It is known that platelets can produce many growth factors for HCC including VEGF [22]. We found statistically significant thrombocytopenia ($p < 0.001$) in the HCC group in contrast to the HCV group; this comes in agreement with the study by Carr et al. who demonstrated that non-cirrhotic HCC patients have lower levels of thrombocytopenia than cirrhotic HCC patients specially in those patients who had larger tumors [23]. In the current study, there was statistically significant anemia ($p < 0.001$) in the HCC group, and this reflect the impact of developing HCC on the deterioration of liver function reserve and deterioration of portal hypertension with increased anemia whether related to malignancy or portal hypertension related to blood loss and reduced platelet count expected in cirrhotic patients with or without malignancy.

In this study, we found highly statistically significantly higher serum AFP in the HCC group in comparison with the HCV infection group; this comes in agreement with Zhang et al. who estimated the performance of AFP in diagnosing HCC and they found that AFP serum level is a good sensitive biomarker to diagnose HCC whether used alone or combined with ultrasound, they found that 400 ng/mL was better than 200 ng/mL as a cutoff value for diagnosis of HCC [24].

Table 5 Linear regression analysis to predict SVR VEGF values in HCV patients

Variables	B	P value	Confidence interval	
			Lower bound	Upper bound
Age	1.94	0.064	− 0.123	4.006
PCR	− 0.000017	0.219	− 0.000045	0.000011

Also, we found that BMI was significantly higher in HCC patients than in HCV group ($p < 0.001$); this comes in agreement with study by Divella et al. who explores the risk of obesity in the development of HCC whether due to the post-viral cirrhosis or in NAFLD [25].

As regards the serum creatinine level, it was significantly higher in the HCC group ($p < 0.001$). Shariff et al. suggested that this increase may be due to the increased metabolic activity that resulted in the increased rate of cellular turnover causing overproduction of carnitine to be sufficient for beta-oxidation and excessive needs of energy [26]. However, reduced serum creatinine in case of chronic liver disease can be due to a 50% decline in hepatic production of creatine; it can be also attributed not only to accumulation of extracellular fluid in the form of edema and ascites which increases the volume of distribution but also to malnutrition and decrease in muscular mass related to recurrent attacks of sepsis affecting satiety of patients [20].

Regarding serum albumin, it was significantly lower in the HCC group also ($p < 0.001$); this result agrees fully with Carr and Guerra who studied the association between serum albumin levels and HCC severity [27]. Albumin production is a marker for hepatocyte function differentiation; low serum albumin level may denote poorly differentiated and more aggressive HCC; on the other hand, normal serum albumin level can be a good indicator for well-differentiated, slowly growing HCCs [28]. However, low albumin level reflects also poor nutrition and could be an indicator of bad prognosis [29]

In this study, we found significantly higher bilirubin in HCC patients compared to the HCV patients' group, and this comes in contact with the study by Carr et al. who also stated that abnormal levels of bilirubin in HCC patients reflect a poor prognosis in contrast to those with normal levels [30].

In our Egyptian cohort of cirrhotic HCV-related and HCC patients, the number is relatively small and genotype 4 is different from other genotypes in other studies, and in addition, other molecular mechanisms may be more involved in our Egyptian patients as alteration of immune system function and inflammatory cytokines.

Conclusion

After successful viral eradication by DAAs, VEGF, the one responsible for both physiological and pathological angiogenesis in HCC, significantly decrease at end of treatment, after 1 month and 3 months of treatment. So, we can conclude that VEGF is not incriminated in the debate of increased incidence of HCC after treatment by DAAs.

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Abbreviations
HCC: Hepatocellular carcinoma; VEGF: Vascular endothelial growth factor; DAAs: Direct-acting antivirals; HCV: Hepatitis C virus; SVR: Sustained virologic response; AFP: Alpha-fetoprotein; HIF-1α: Hypoxia-inducible factor 1 alpha; STAT3: Signal transducer and activator of transcription 3; HBV: Hepatitis B virus; CBC: Complete blood count; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; PCR: Polymerase chain reaction

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References
1. Yang JD, Hainaut P, Gores GJ, Amadou A, Plymoth A, Roberts LR (2019) A global view of hepatocellular carcinoma: trends, risk, prevention and management. Nat. Rev. Gastroenterol. Hepatol. 16:589–604
2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A (2018) Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J. Clin. 68:394–424
3. Axley P, Ahmed Z, Ravi S, Singal AK (2018) Hepatitis C virus and hepatocellular carcinoma: a narrative review. J. Clin. Transl. Hepatol. 6:79–84
4. Hsu SJ, Yang SS, Kao JH (2020) Risk of hepatocellular carcinoma development after hepatitis C virus eradicated by direct-acting antivirals: fact or fiction? J. Formos. Med. Assoc 119(Pt 1):3–11.
5. Ioannou GN, Green PK, Beste LA, Mun EJ, Kerr KF, Berry K (2018) Development of models estimating the risk of hepatocellular carcinoma after antiviral treatment for hepatitis C. J. Hepatol. 69:1088–1098
6. Ioannou GN, Beste LA, Green PK, Singal AG, Tapper EB, Waljee AK et al (2019) Increased risk for hepatocellular carcinoma persists up to 10 years after HCV eradication in patients with baseline cirrhosis or high FIB-4 scores. Gastroenterology 157:1264–1278 e4
7. Carrat F, Fontaine H, Dorival C, Simony M, Diallo A, Hezode C et al (2019) Clinical outcomes in patients with chronic hepatitis C after direct-acting antiviral treatment: a prospective cohort study. Lancet 393:1453–1464
8. Baumert TF, Juhling F, Ono A, Hoshida Y (2017) Hepatitis C-related hepatocellular carcinoma in the era of new generation antivirals. BMC Med. 15:52
9. Waziry R, Hajarizadeh B, Grebely J, Amin J, Law M, Danta M et al (2017) Hepatocellularcarcinoma risk following direct-acting antiviral HCV therapy: a systematic review, meta-analyses, andmeta-regression. J. Hepatol. 67:1204–1212
10. Capurro M, Filmus J (2005) Glypican-3 as a serum marker for hepatocellular carcinoma. Cancer Res 65(1):372–373
11. Hamdy MN, Shaheen KY, Awad MA, Barakat EMF, Shalaby SI, Gupta N et al (2020) Vascular endothelial growth factor (VEGF) as a biochemical marker for the diagnosis of hepatocellular carcinoma (HCC). Clinical Practice. 17(1): 1441–1453
12. Zhu AX, Duda DG, Sahani DV, Jain RK (2011) HCC and angiogenesis: possible targets and future directions. Nat. Rev. Clin. Oncol. 8:292–301
13. Hassan M, Selimovic D, Ghozlan H, Abdel-kader O (2009) Hepatitis C virus core protein triggers hepatic angiogenesis by a mechanism including multiple pathways. Hepatology 49:1469–1482
14. Kanda T, Steele R, Ray R, Ray RB (2008) Hepatitis C virus core protein augments androgen receptor-mediated signaling. J. Virol. 82:11066–11072
15. Mukozu T, Nagal H, Matsui D, Kanekawa T, Sumino Y (2013) Serum VEGF as a tumor marker in patients with HCV-related liver cirrhosis and hepatocellular carcinoma. ANTICANCER RESEARCH. 33:1013–1022
16. Villani R, Facciorusso A, Bellanti F, Tamborra R, Piscazzi A, Landriscina M et al (2016) DAAs rapidly reduce inflammation but increase serum VEGF level: a rationale for tumor risk during anti-HCV treatment. PLoS ONE. 11(12): e0167934
17. Roche B, Coilly A, Duclos-Vallee JC, Samuel D (2018) The impact of treatment of hepatitis C with DAAs on the occurrence of HCC. Liver Int. 38(1):130–145
18. Debes JD, Tilborg MV, Groothuismink ZMA, Hansen BE, Wiesch JSZ, Felden JV et al (2018) Levels of cytokines in serum associate with development of hepatocellular carcinoma in patients with HCV infection treated with direct-acting antivirals. Gastroenterology. 154(3):515–517
19. Faillaci F, Marzi L, Critelli R, Milosa F, Schepis F, Turola E et al (2018) Liver angiopoietin-2 is a key predictor of *de novo* or recurrent hepatocellular cancer after hepatitis C virus direct-acting antivirals. Hepatology. 68(3):1010–1024
20. Takabatake T, Ohta H, Ishida Y, Hara H, Ushiogi Y, Hattori N (1988) Low serum creatinine levels in severe hepatic disease. Arch Intern Med. 148: 1313–1315
21. Cheung MCM, Walker AJ, Hudson BE, Verma S, McLauchlan J, David J, Mutimer DJ et al (2016) Outcomes after successful direct-acting antiviral therapy for patients with chronic hepatitis C and decompensated cirrhosis. J Hepatol. 65(4):741–747
22. Nalesnik MA, Michalopoulos GK (2012) Growth factor pathways in development and progression of hepatocellular carcinoma. Front Biosci (School Educ) 4:1487–1515
23. Carr BI, Guerra V (2013) Thrombocytosis and hepatocellular carcinoma. Dig Dis Sci. 58(6):1790–1796
24. Zhang J, Chen G, Zhang P, Zhang J, Li X, Gan D et al (2020) The threshold of alphafetoprotein (AFP) for the diagnosis of hepatocellular carcinoma: a systematic review and meta-analysis. PLoS ONE 15(2):e0228857
25. Divella R, Mazzocca A, Daniele A, Sabbà C, Paradiso A (2019) Obesity, non-alcoholic fatty liver disease and adipocytokines network in promotion of cancer. Int J Biol Sci. 15(3):610–616
26. Shariff MI, Gomaa AI, Cox IJ, Patel M, Williams HR, Crossey MM et al (2011) Urinary metabolic biomarkers of hepatocellular carcinoma in an Egyptian population: a validation study. J Proteome Res. 10:1828–1836
27. Carr BI, Guerra V (2017) Serum albumin levels in relation to tumor parameters in hepatocellular carcinoma patients. Int J Biol Markers 32(4):e391–e396
28. Hernandez-Gea V, Toffanin S, Friedman SL, Llovet JM (2013) Role of the microenvironment in the pathogenesis and treatment of hepatocellular carcinoma. Gastroenterology. 144(3):512–527
29. Schütte K, Tippelt B, Schulz C (2015) Malnutrition is a prognostic factor in patients with hepatocellular carcinoma (HCC). Clin Nutr. 34:1122–1127
30. Carr BI, Guerra V, Giannini EG, Farinati F, Ciccarese F, Rapaccini GL et al (2014) Association of abnormal plasma bilirubin with aggressive HCC phenotype. Semin Oncol. 41(2):252–258

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