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ORIGINAL RESEARCH

Prognostic Significance of VEGF and HIF-1 α in Hepatocellular Carcinoma Patients Receiving Sorafenib Versus Metformin Sorafenib Combination

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Abstract

Background Hepatocellular carcinoma (HCC) is a major health problem. HCC burden has been increasing in Egypt in the past 10 years. Most HCC cases are diagnosed at an advanced stage with limited treatment options. Sorafenib is the standard therapy for advanced HCC, but the effectiveness is not satisfied. Metformin may decrease the risk of HCC development in diabetic patients, reduces tumor invasion, and augments sensitivity to sorafenib; however, safety and efficacy of combined treatment are still unclear. As HCC is characterized by high vascularity, and vascular endothelial growth factor (VEGF) plays an important role in vascularization, many studies questioned if VEGF and HIF-1 α could offer information about HCC response to sorafenib. We conducted this study to assess the benefits from adding metformin to HCC treatment, and appraise the role of VEGF and HIF-1 α in HCC prognosis.

Method This was a prospective, randomized study in which 80 advanced measurable patients consecutively treated with sorafenib plus metformin (arm A) or sorafenib alone (arm B), prognostic value of plasma, and tissue levels of VEGF and HIF-1 α were evaluated.

Results We enrolled 61 men and 19 women with a median age of 60 years (range 49–68 years). Fifty-seven patients had Child–Pugh A while 23 had early B, the most common etiology of liver disease was hepatitis C (86%). Sixty percent of patients were diabetic. No significant difference was detected between arm A and arm B regarding response to treatment ($p = 0.5$), time to disease progression ($p = 0.3$), or overall survival ($p = 0.6$). Low VEGF and HIF-1 α plasma levels were significantly associated with better treatment response ($p < 0.001$ for both), and higher OS ($p < 0.001$). Patients with high expressions of VEGF and HIF in HCC tissue had significantly poor treatment outcome ($p < 0.001$, $p = 0.03$, respectively), and poor OS ($p < 0.001$, $p < 0.001$, respectively).

Conclusions No superior efficacy of adding metformin to sorafenib in HCC treatment. VEGF and HIF-1 α had promising prognostic value in HCC.

Keywords Hepatocellular carcinoma · VEGF · HIF-1 α · Sorafenib · Metformin

Introduction

Hepatocellular carcinoma (HCC) is the most frequent primary liver tumor [1]. The frequency of HCC is rising worldwide [2], and in Egypt, particularly in the previous decade [3]. HCC may be cured via transplantation if discovered early; however, nearly all cases are diagnosed in late stage, leading to restriction of treatment options. Medical treatment is still one of the major problems in oncology since HCC is mostly chemo-resistant tumor, and no systemic drug was offered for patients with advanced stage until 2007, when sorafenib, which is a multikinase inhibits the VEGFR, the platelet-derived growth factor receptor, and Raf kinases, has been proved to increase median overall survival (OS) in HCC [4, 5]. Llovet et al. [4]

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revealed better OS in patients received sorafenib compared with a placebo group ($p = 0.00058$). Now, sorafenib is the optimal drug for advanced HCC; however, the effectiveness is still dissatisfied [6]. Metformin is a drug approved for management of type 2 diabetes mellitus. In HCC, metformin enhances insulin sensitivity, reduces gluconeogenesis in liver, and reduces glycogenolysis [7]. The rationale for metformin usage in this study arises from preceding trials results, as metformin can inhibit tumor formation through the LKB1-AMPK pathway [8, 9]. Previous studies recommended that the risk of HCC in type 2 diabetes can be reduced by metformin therapy; moreover, metformin delays HCC invasion and augments the sensitivity to sorafenib [7, 10], but the safety and efficiency of this combined therapy remain uncertain.

HCC is an extremely vascular cancer, and vascular endothelial growth factor (VEGF) has significant roles in vascularization [11], also it is a master controller of angiogenesis, which is a nonstop formation of new blood vessels that are essential for cancer growth and survival, and that lead to significant tumor development [12]. There are multiple family members of VEGF and each of them has specific functions. VEGF activates receptors related to the proliferation of tumor cells [13], so VEGF-targeted agents may be effective in treatment of advanced HCC. Several clinical studies questioned whether VEGF level in blood samples could give sensitive information about HCC response to sorafenib treatment; however, the results of these studies are still not conclusive [14]. Also multiple trials evaluated the relation between VEGF tissue overexpression with patients' outcome in HCC, but results were conflicting; a meta-analysis of 14 studies that examined the relation between VEGF overexpression in tumor tissue and survival in patients with HCC suggested that VEGF overexpression had an unfavorable impact on overall survival (OS), but not disease-free survival (DFS).

HCC and other solid tumors are characterized by tissue hypoxia, especially when the tumor grows quickly and angiogenesis fails to stand with the speed of tumor growth, and this hypoxic environment leads to pro-survival reactions in HCC cells, leading to angiogenesis, tumor invasion, and metastasis [15]. Hypoxia inducible factor-1 (HIF-1) is the master mediator of cell response to hypoxia [16].

The HIF-1 complex is a transcription factor for some genes in carcinogenesis including angiogenesis, apoptosis, cell proliferation, and glucose metabolism [17, 18]. Vascular endothelial growth factor (VEGF) is one of the major target genes for HIF-1 α that leads to angiogenesis [19], as under hypoxia; HIF-1 α causes activation of VEGF pathway.

Up till now, the sorafenib which is a multikinase inhibitor is still the only approved treatment for advanced HCC, and it has been demonstrated that the mechanisms that account for the anti-angiogenic efficiency of sorafenib are related to its inhibitory effect on HIF-1 α and VEGF proteins expression, leading to a decrease in vascularization of HCC [20].

Various trials demonstrated that serum HIF-1 α expression is significantly high in HCC than in benign liver disease, suggesting that circulating HIF-1 α level is a new biomarker for HCC diagnosis and prognosis [21–23]. Moreover, many trials reported that higher level of HIF-1 α tissue expression might indicate a poorer prognosis in HCC, and HIF-1 α could be used as a useful biomarker for the HCC prognosis. However, the conclusion is still uncertain due to the limitations of the included studies, and more studies to evaluate the prognostic significance of HIF-1 α in HCC are recommended [24, 25].

Aiming to compare the efficacy, safety, and prognosis of treatment with sorafenib plus metformin versus sorafenib alone in patients with advanced HCC in relation to serum VEGF and HIF-1 α concentrations, we designed the study.

Patients and Method

This is a prospective, randomized controlled study, comparing the treatment outcome, time to disease progression (TDP), overall survival (OS), and toxicity in patients with advanced measurable HCC who received combination of sorafenib 400 mg twice daily plus metformin 500 mg twice daily (arm A) versus sorafenib alone 400 mg twice daily (arm B). Crossover not allowed from one arm to another.

Safety profile was assessed in all patients receiving at least one cycle of studied drugs, using the National Cancer Institute's Common Terminology Criteria for adverse events (CTCAE v4.03: June 14, 2010).

Though treatment was received in a continuous manner, treatment period was divided into 8-week cycles for recording information and tumor evaluation which was done by computed tomography or magnetic resonance imaging; also patients visited the clinic every 4 weeks and estimated for compliance, and safety. Safety evaluations included records of adverse effects, laboratory tests (hematologic and biochemical analyses), physical examination, and assessment of vital signs.

The treatment with sorafenib was continued until disease progression, unacceptable toxicity, or death. Disease progression was evaluated using RECIST response criteria [26].

We explored the prognostic value of serum VEGF and HIF-1 α level in 80 patients with advanced HCC, serum VEGF and HIF-1 α concentration were measured using enzyme-linked immunosorbent assay. Furthermore, VEGF and HIF-1 α expression in HCC tissue were assessed in 30 HCC patients only (who accepted to do liver biopsy) by immunohistochemistry.

This study was approved by Zagazig University Institutional Review Board (IRB), and carried out from December 2014 to January 2016 at Zagazig University and El Mabara Hospitals.

Eligible for Study

Inclusion Criteria

- -Age: 18 years and above.
- -Patients with histologically or radiologically proved hepatocellular carcinoma (HCC).
- -Patients should have as a minimum one lesion that exactly measured in at least one dimension as said by RECIST (Response Evaluation Criteria in Solid Tumors).
- -Patients who have ECOG performance status of 2 or less (at least, being up and about equal to or greater than 50% of waking hours), Child–Pugh liver function class A or early B (based on total bilirubin, serum albumin, PT/INR, ascites, hepatic encephalopathy), adequate hematologic function, adequate hepatic, and renal function.

Exclusion Criteria

- -Previous or concomitant cancer with different primary site or histology from HCC
- -Renal failure requiring hemodialysis or peritoneal dialysis
- -History of cardiac disease
- -Active clinically serious infections
- -Significant gastrointestinal bleeding in 30 days before the study
- -Or, received prior molecular targeted treatment or any other systemic therapy

Methods

ELISA Method Ten milliliters of patient blood was taken and kept at room temperature for over 30 min and centrifuged at 2000*g* for 10 min then kept at − 70 °C. The level of serum HIF-1 α and VEGF (R&D system, Abingdon UK, and ADL Biotech Dev Co., USA) was used to detect by ELISA in accordance with the manufacturer's instruction. During the procedure, the plate was washed according to the routine ELISA method concentrations that are calculated by a standard curve generated with specific standards provided by the manufacture. Intra- and inter-assay variations were lower than 10%.

Immunohistochemical Staining Immunohistochemical staining was carried out using streptavidin–biotin immune-peroxidase technique [27]. The slides were incubated with rabbit polyclonal anti-HIF-1alpha antibody—ChIP Grade ab2185 was used at a dilution of 1:100 and anti-VEGFA antibody ab46154 diluted 1/200 at 4 °C overnight (Abcam, Cambridge, MA, USA).

Evaluation of Immunohistochemical Expression of HIF-1 α

The protein levels of HIF-1 α a were scored according to the number of cells exhibiting the cytoplasmic and nuclear staining using the following classification system: I, no staining; II, nuclear staining in, 10% of cells and/or with weak cytoplasmic staining; III, nuclear staining in 10–50% of cells and/or with distinct cytoplasmic staining; IV, nuclear staining in 0.50% of cells and/or with strong cytoplasmic staining. In the following analysis, cases of scores I and II were considered low expression patterns while the remaining cases were considered as high expression patterns [28–31]. Sections were scored semi-quantitatively as follows [32]: (negative), 0% immunoreactive cells; + ≦ 5% immunoreactive cells; ++ > 5–50% immunoreactive cells; +++ ≧ 50 immunoreactive cells. For statistical purposes, cases with scores 0 and + were considered low expression and those with scores ++ and +++ were considered high expression. Regarding HIF-1 α scoring, low expression was defined as < 10% of cells exhibiting nuclear staining and/or cytoplasmic staining. High expression was determined when ≥ 10% of cells exhibited nuclear staining and/or distinct cytoplasmic staining [28].

Evaluation of Immunohistochemical Expression of VEGF

The VEGF positive staining had a cytoplasmic localization. The percentage of positive VEGF cells was assessed by examining 10 microscopic fields at high magnification (× 400) from each section. The IHC expression of VEGF was evaluated/graded using a semi quantitative score, according to the sum of two parameters: the percentage of positive cells and the intensity of immunostaining.

- The percentage of positive cells:

  0 = 0% immune-positive cells; 1 = < 25% positive cells; 2 = 26–50% positive cells; 3 = > 50% positive cells

- The intensity of immunostaining:

  0 = negative immunoreaction; 1 = weak intensity; 2 = moderate intensity; 3 = strong intensity

By summing up the two parameters we obtained a final score that varies between 0 and 6. In our study we considered:

- Negative immunoreaction (−) for a score between 0 and 2
- Weakly positive immunoreaction (+) for a score between 3 and 4
- Intensely positive immunoreaction (++) for a score between 5 and 6

The immune-histochemical reactions for VEGF were applied for all the cases of liver cancer included in the study. We identified the expression of the antibody both in the tumor and surrounding hepatic tissue [33].

Statistical Analysis Descriptive data were reported as median with range for continuous variables, and absolute and relative frequencies for categorical variables. Time to disease progression (TDP) is from randomization to radiological disease progression. Subjects still alive at the time of analysis were censored at their last date of last contact. Overall survival (OS): from randomization to death due to any cause. Kaplan-Meier method estimated the TDP and OS, and their 95% CI were compared with the log-rank test. SPSS statistical software version 22 (Chicago, IL, USA) was used for all statistical analyses and a p value < 0.05 was considered statistically significant.

Results

We included 80 advanced HCC patients; 61 men (76.3%) and 19 women (23.7%) with a median age of 60 years (range 49–68 years). 71.3% (57/80) of patients had Child–Pugh A and 28.7% (23/80) of patients had early B Child–Pugh, from December 2014 to January 2016 at Zagazig University and El Mabara Hospitals. The most common etiologies of liver disease were hepatitis C (86%) and hepatitis B (15%). Forty-eight patients (60%) were diabetic and 24 (30%) were controlled hypertensive. Advanced HCC patients were consecutively randomized to be treated with sorafenib plus metformin (arm A) or sorafenib alone (arm B).

The characteristics of the all 80 patients are shown in Table 1.

Patients in both arms were balanced regarding age, sex, diabetes, AFP level, and HCV and HBV infection (Table 2).

Treatment Outcome and Survival of Both Arms

Treatment with (arm A) was associated with overall response rate (ORR) 52.5% compared with 55% in (arm B) ($p = 0.5$).

In arm A, 19/40 (47.5%) of patients had progressive disease (PD), 42.5% had stable disease (SD), and 10% had partial response (PR), while in arm B, 45% had PD, 40% had SD, and 15% had PR ($p = 0.79$).

For all patients, the median TDP was 8 months (95% CI 6.4–9.5) and median OS was 10 months (95% CI 10.6–12.3).

We found patients with hypertension and positive HCV infection had a significant longer TDP than normotensive and negative HCV infection patients ($p = 0.04$ and 0.01, respectively), but no difference as regards age, sex, diabetes, HBV infection, ascites, PS, AFP levels, or number of tumor lesions.

Table 1 All 80 patients' characteristics

Characteristic	No. patients (%)
Median age, years (range)	60 (49–68)
Sex	
Male	61 (76.3)
Female	19 (23.7)
HCV infection	69 (86.3)
HBV infection	12 (15)
DM	48 (60)
HTN	24 (30)
Ascitis	
No	57 (71.3)
Minimal	9 (11.3)
Mild	14 (17.5)
PS	
0–1	55 (68.8)
2	25 (31.3)
Child	
A	57 (71.3)
Early B	23 (28.7)

Patients treated with (arm A) had a mean TDP of 8.7 ± 0.8 months (95% CI 6.9–10.3) compared with 8.2 ± 0.4 months (95% CI 7.4–8.9) for patients in (arm B) ($p =$

Table 2 The patients' characteristics of both arms

Variable	Sorafenib + metformin (arm A) No. (%)	Sorafenib alone (arm B) No. (%)	p value
Age			
< 60	17 (47.2%)	19 (52.8%)	0.4
≥ 60	23 (52.3%)	21 (47.7%)	
Sex			
Male	30 (49.2%)	31 (50.8%)	0.5
Female	10 (52.6%)	9 (47.4%)	
AFP			
< 400	15 (48.4%)	16 (51.6%)	0.5
≥ 400	25 (51%)	24 (49%)	
HCV			
Yes	34 (49.3)	35 (50.7)	0.5
No	6 (54.5)	5 (45.5)	
HBV			
Yes	5 (41.7%)	7 (58.3%)	0.3
No	35 (51.5%)	33 (48.5%)	
Diabetes			
Yes	22 (45.8%)	26 (54.2%)	0.2
No	18 (56.2%)	14 (43.8%)	
Hypertension			
Yes	9 (64%)	5 (36%)	0.1
No	13 (45%)	16 (55%)	

Table 3 Outcomes related to sorafenib plus metformin (arm A) and sorafenib alone (arm B)

ARM		Time to disease progression (TDP)			Overall survival (OS)		
	No. patients	No. events	Mean TDP ± SD (95% CI)	*p*	No. events	Mean OS ± SD (95% CI)	*p*
A	40	10	8.7 ± 0.8 months (95% CI 6.9–10.3)	0.3	20	10.775 ± 0.855 (95% CI 9.1–12.4)	0.6
B	40	9	8.2 ± 0.4 months (95% CI 7.4–8.9)		19	12.205 ± 0.968 (95% CI 10.3–14.103)	

Arm A: sorafenib + metformin

Arm B: sorafenib alone

0.3), while, mean OS of 10.775 ± 0.855 (95% CI 9.1–12.4) and 12.2 ± 0.96 (95% CI 10.3–14.103) for arm A and arm B, respectively ($p = 0.6$: Table 3 and Figs. 1 and 2).

Safety Assessment

Adverse events that were reported for both arms were mainly grade 1 or 2 in severity (gastrointestinal, or dermatologic in nature), there were no grade 4 drug-related adverse events except one case in arm A with grade 4 hypertension (Table 4).

The rate of dose reductions or discontinuation of treatment due to adverse events (summation of grades 3 and 4) was 15% in arm A versus 22.5% in arm B. The most frequent adverse events were diarrhea (20%, 17.5% in arms A and B consecutively), anorexia (20% versus 10% in arm A versus arm B), fatigue (15% in arm A and 17.5% in arm B), and alopecia (17.5% in arm A and 25% in arm B).

Plasma VEGF and HIF-1 α Levels by ELISA

Plasma VEGF and HIF-1 α levels were assessed at different cutoff values. For VEGF, at a cutoff value of 489 pg/mL, the sensitivity was 92% and specificity was 91% for HCC with an area under the receiver operating characteristic curve (AUROC) of 0.89. For HIF-1 α, at a cutoff value of 186 pg/mL, the sensitivity was 89% and specificity was 81% for HCC with an area under the receiver operating characteristic curve of 0.8.

No significant difference between the mean plasma VEGF levels in both HCC groups (493.00 ± 0.5 pg/mL and 546.48 ± 263.4 pg/mL for arm A and B, respectively, $p = 0.3$), as well as for HIF-1 α plasma levels (1.4 ± 0.49 pg/mL and 1.47 ± 0.5 pg/mL for arm A and B, respectively, $p = 0.5$). The plasma VEGF and HIF-1 α levels were significantly correlated with the maximum size of the tumors ($p < 0.001$ for both), TLC ($p = 0.02$ for both) and bilirubin ($p = 0.003$ and 0.01, respectively, Table 5).

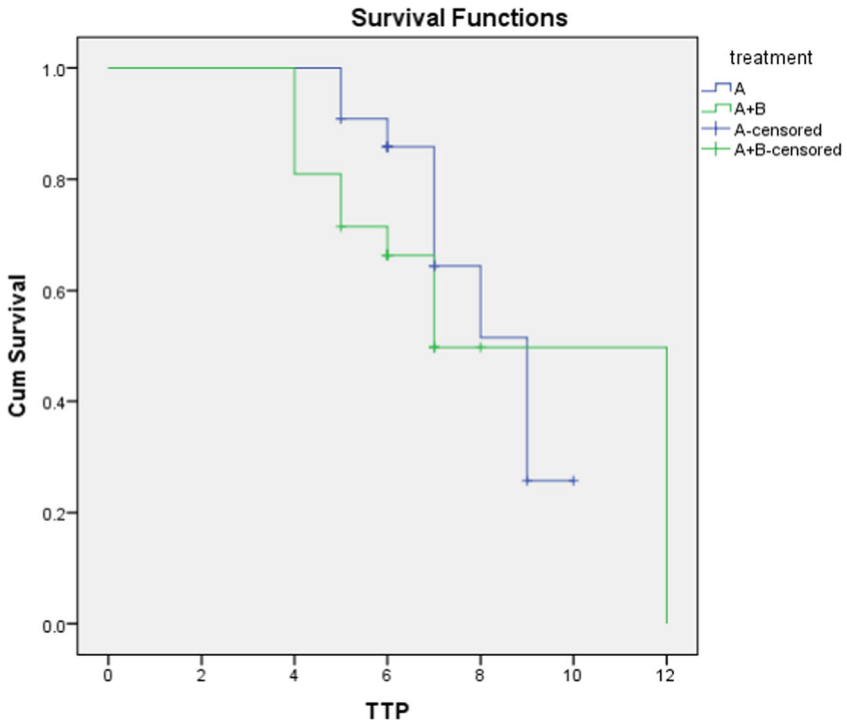

Fig. 1 Kaplan-Meier analysis of time to disease progression (TDP) of both arms ($p = 0.3$)

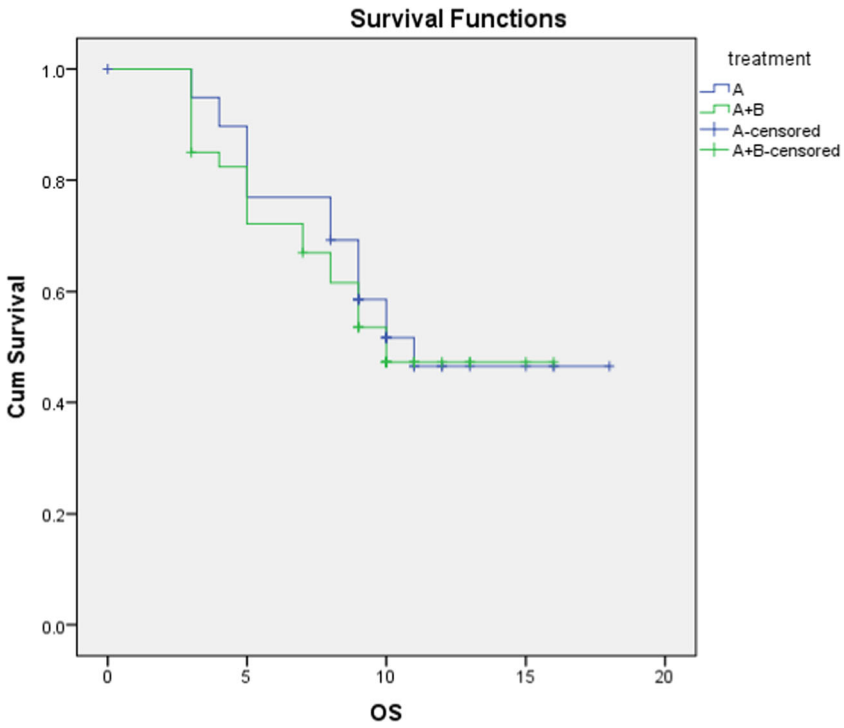

Fig. 2 Kaplan-Meier analysis of overall survival (OS) of both arms ($p = 0.6$)

Table 4 Incidence of drug-related adverse events of sorafenib plus metformin (arm A) and sorafenib alone (arm B)

Arm (A)							Arm (B)						*p* value
Toxicity grade	G0	G1	G2	G3	G4	Any grade	G0	G1	G2	G3	G4	Any grade	
Diarrhea	32 (80%)	6 (15%)	1 (2.5%)	1 (2.5%)	0 (0%)	8 (20%)	33 (82.5%)	4 (10%)	2 (5%)	1 (2.5%)	0 (0%)	7 (17.5%)	0.86
Nausea	35 (87.5%)	2 (5%)	2 (5%)	1 (2.5%)	0 (0%)	5 (12.5%)	37 (92.5%)	1 (2.5%)	1 (2.5%)	1 (2.5%)	0 (0%)	3 (7.5%)	0.86
Abdominal pain	36 (90%)	3 (7.5%)	1 (2.5%)	0 (0%)	0 (0%)	4 (10%)	33 (82.5%)	4 (10%)	3 (7.5%)	0 (0%)	0 (0%)	7 (17.5%)	0.52
Anorexia	32 (80%)	3 (7.5%)	3 (7.5%)	2 (5%)	0 (0%)	8 (20%)	36 (90%)	2 (5%)	1 (2.5%)	1 (2.5%)	0 (0%)	4 (10%)	0.62
Fatigue	34 (85%)	4 (10%)	2 (5%)	0 (0%)	0 (0%)	6 (15%)	33 (82.5%)	5 (12.5%)	2 (5%)	0 (0%)	0 (0%)	7 (17.5%)	0.93
Weight loss	37 (92.5%)	2 (5%)	1 (2.5%)	0 (0%)	0 (0%)	3 (7.5%)	36 (90%)	2 (5%)	1 (2.5%)	1 (2.5%)	0 (0%)	4 (10%)	0.79
Hand and foot skin reaction	37 (92.5%)	2 (5%)	1 (2.5%)	0 (0%)	0 (0%)	3 (7.5%)	35 (87.5%)	3 (7.5%)	1 (2.5%)	1 (2.5%)	0 (0%)	5 (12.5%)	0.7
Alopecia	33 (82.5%)	4 (10%)	3 (7.5%)	0 (0%)	0 (0%)	7 (17.5%)	30 (75%)	4 (10%)	6 (15%)	0 (0%)	0 (0%)	10 (25%)	0.56
Pruritus	38 (95%)	1 (2.5%)	1 (2.5%)	0 (0%)	0 (0%)	2 (5%)	37 (92.5%)	2 (5%)	1 (2.5%)	0 (0%)	0 (0%)	3 (7.55)	0.84
Bleeding	37 (92.5%)	2 (5%)	1 (2.5%)	0 (0%)	0 (0%)	3 (7.5%)	37 (92.5)	1 (2.5%)	1 (2.5%)	1 (2.5%)	0 (0%)	3 (7.5%)	0.72
Thrombocytopenia	36 (9%)	2 (5%)	2 (5%)	0 (0%)	0 0%	4 (10%)	36 (9%)	2 (5%)	1 (2.5%)	1 (2.5%)	0 (0%)	4 (10%)	0.63
Liver dysfunction	38 (95%)	1 (2.5%)	1 (2.5%)	0 (0%)	0 (0%)	2 (5%)	37 (92.5%)	1 (2.5%)	1 (2.5%)	1 (2.5%)	0 (0%)	3 (7.5%)	0.73
Hypertension	36 (9%)	1 (2.5%)	1 (2.5%)	1 (2.5%)	1 (2.5%)	4 (10%)	38 (95%)	0 (0%)	1 (2.5%)	1 (2.5%)	0 (0%)	2 (5%)	0.58

Listed are adverse events, as defined by the National Cancer Institute Common Terminology Criteria that occurred in either study group

Table 5 Correlation between plasma VEGF, HIF-1 alpha levels, and clinic-pathological characteristics of the all the patients

	Plasma VEGF								
Correlation	Age	TLC	HB	PLT	Albumin	Bilirubin	INR	AFP	T size
Pearson correlation (*r*)	− 0.12	− 0.24	0.04	− 0.17	− 0.05	0.3	0.2	− 0.09	0.4
p value	0.2	0.02	0.6	0.13	0.6	0.003	0.04	0.3	< 0.001
Plasma HIF-1 alpha									
Correlation	AGE	TLC	HB	PLT	ALBUMIN	BILIRUBIN	INR	AFP	T size
Pearson correlation (*r*)	− 0.04	− 0.25	0.05	− 0.14	− 0.07	0.27	0.2	− 0.025	0.48
p value	0.66	0.02	0.6	0.21	0.5	0.01	0.05	0.8	< 0.00

Low VEGF and HIF-1 α plasma levels were significantly associated with better treatment response ($p < 0.001$), higher OS ($p < 0.001$), TDP ($p < 0.001$) for both, and with HCV infection for HIF-1 α plasma level only ($p = 0.03$; Table 6).

Tissue VEGF and HIF-1 α Expression by Immunohistochemistry

True cut biopsy was done for 30 HCC patients only (who had accepted) and the tissue expression levels of VEGF and HIF-1 α were evaluated by immunohistochemistry, high tissue expression of VEGF was detected in 21/30 (70%) patients, while high tissue expression of HIF-1 α was detected in 18/30 (60%) patients (Figs. 3 and 4).

High tissue expression of VEGF was significantly associated with poor response, as from 21 high expression patients; 15 patients had PD, and 6 had SD ($p < 0.001$), and significantly low overall survival ($p < 0.001$).

Patients with high tissue expression of HIF-1 α had significantly poor response ($p = 0.03$) and poor survival ($p < 0.001$) compared with those with low expression (Table 7).

Discussion

Hepatocellular carcinoma (HCC) is one of the most frequent malignancy worldwide, and the top three in both incidence and mortality [34].

Despite advanced diagnosis and treatment, incidence and mortality are still rising. HCC is a very aggressive cancer and the diagnosis of HCC is often occurred in advanced stages when patients become symptomatic and have some degree of liver impairment. At this late stage, there is no effective treatment that leads to improve survival. The oral multitargeted tyrosine kinase inhibitor sorafenib has become the standard treatment for advanced HCC. Sorafenib blocks the activity of Raf serine/threonine kinase isoforms, vascular endothelial growth factor receptors 2 and 3, platelet-derived growth factors receptor β, c-KIT, FLT-3, and RET, to inhibit tumor angiogenesis and proliferation [2]. Until now, sorafenib is the first choice in patients with advanced HCC and preserved liver function [1, 4]. Type 2 diabetes is a significant risk factor for the development of malignancies, including HCC [35], some reported that HCC incidence is significantly increased with elevated glycated hemoglobin levels [36], while others have reported

Table 6 Relation between plasma VEGF, HIF-1 alpha levels, and disease outcome

Variable	VEGF plasma level		*p* value	HIF-1 α plasma level		*p* value
	Low	High		Low	High	
ARM						
A	20	20		21	19	0.3
B	21	19	0.5	24	16	
Response						
PR + SD	40	1	< 0.001	32	1	< 0.001
PD	3	36		3	34	
Median OS (months)	Not reached	5	< 0.001	Not reached	5	< 0.001
Mean OS ± SD (months)	16.9 ± 0.5	6.3 ± 0.4		15.9 ± 0.6	6.3 ± 0.4	
Median TDP (months)	Not reached	3	< 0.001	Not reached	3	< 0.001
Mean TDP ± SD (months)	11.8 ± 0.19	2.9 ± 0.2		11.3 ± 0.3	2.8 ± 0.2	

Arm A: sorafenib + metformin

Arm B: sorafenib alone

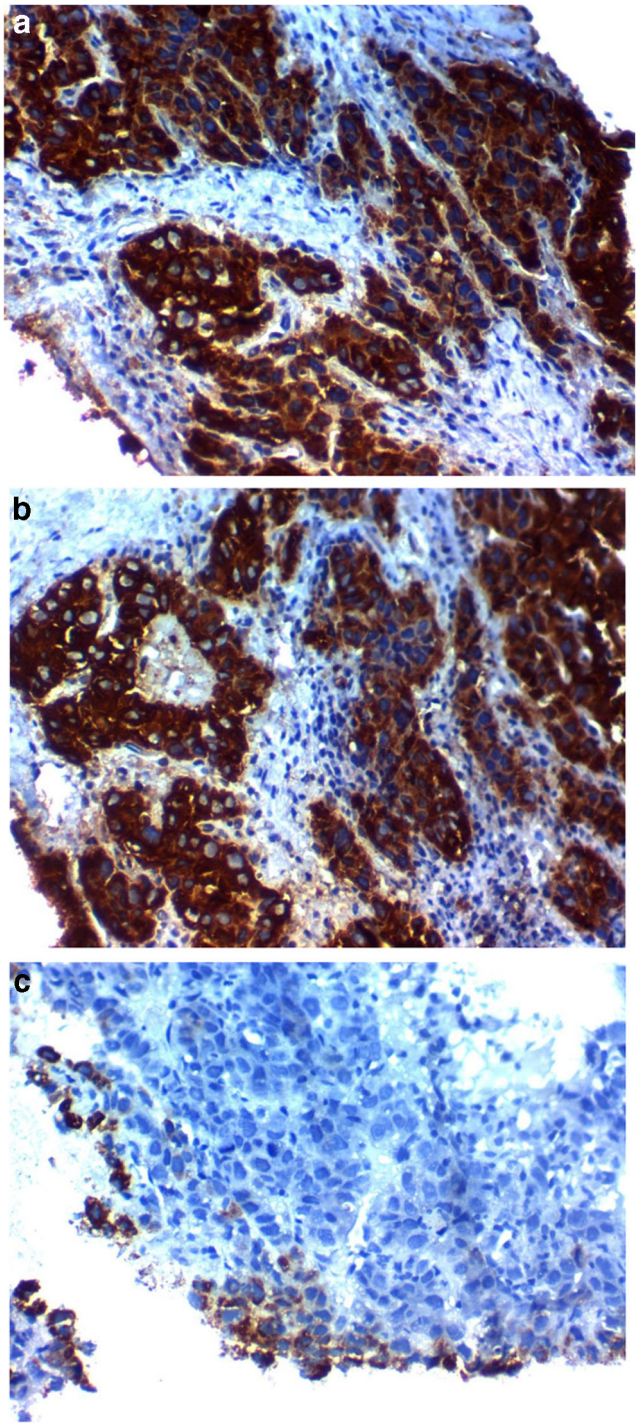

Fig. 3 Immunohistochemical expression of VEGF in hepatocellular carcinoma (HCC). **a** High expression in the cytoplasm of HCC cells × 400. **b** High expression in the cytoplasm of HCC cells × 400. **c** Low expression in the cytoplasm of HCC cells × 400. **a**–**c** The original magnification was × 400

conflicting data on this, and others reported that metformin has a chemo-preventive effect for HCC among patients with insulin resistance [37–43].

We found no significant benefit for adding metformin to sorafenib either in treatment response or survival, as patients of arm A had a mean TDP of 8.7 ± 0.8 months (95% CI 6.9–10.3) compared with 8.2 ± 0.4 months (95% CI 7.4–8.9) for arm B ($p = 0.3$), while mean OS of 10.775 ± 0.855 (95% CI 9.1–12.4) and 12.2 ± 0.96 (95% CI 10.3–14.103) for arm A and arm B, respectively, and this was in harmony with results from Mamatha Bha et al. (2015) [42] who demonstrated no survival benefit to the use of metformin in diabetic patients with HCC with a HR (95% CI 1.0 (0.8–1.3)).

But our results were in disagreement to the results of Casadei Gardini A et al. (2017) [43] who reported increased tumor aggressiveness and resistance to sorafenib in patients treated with metformin chronically and they suggested that may be due to molecular alterations in transporter genes or transcription factors involved in molecular action and pharmacokinetics leading to different response to these drugs' combination. In their study, 280 HCC patients consecutively treated with sorafenib twice daily between March 2008 and August 2016 were included in the study. Metformin with sorafenib was associated with a median PFS of 1.9 months (95% CI 1.8–2.3) compared with 3.7 months (95% CI 3.1–4.6) for patients without metformin ($p < 0.0001$), and a median OS was 6.6 months (95% CI 4.6–8.7) in patients treated with metformin plus sorafenib compared with 10.8 months (95% CI 9.0–13.1) for patients without metformin ($p = 0.0001$). Also patients treated with metformin showed a higher percentage of progression at the first CT re-evaluation.

While in a meta-analysis of 11 trials containing 3452 HCC patients, they revealed that usage of metformin significantly decreased mortality by 41% (HR = 0.59; 95% CI, 0.42–0.83; $p = 0.002$) [44]. This difference in results from ours may be due to heterogeneity in patients' characteristics, tumor etiology, tumor severity, different health states, and prior treatments. So, more prospective trials are needed to establish the beneficial effect of metformin in cancer treatment.

Angiogenesis contributes to the significant cancer growth, including HCC [11]. Vascular endothelial growth factor (VEGF) is a master regulator of angiogenesis in normal and malignant tissues. There are various family members of VEGF and each of them exerts biological functions by binding to different receptors. VEGF plays important roles in proliferation of endothelial cells, leading to neovascularization around and within tumor tissues. With regard to the important roles of VEGF in HCC, VEGF-targeted agents may be effective in the treatment of advanced disease. Sorafenib is a small molecular tyrosine kinase inhibitor blocking the synthesis of important cellular factors (e.g., VEGF) in the regulation of angiogenesis and progression of HCC [11–14]. As the level of VEGF can be measured in blood samples, several clinical studies questioned whether VEGF could provide sensitive information about HCC response to sorafenib; however, the results of these studies are not certain and conflicting [12].

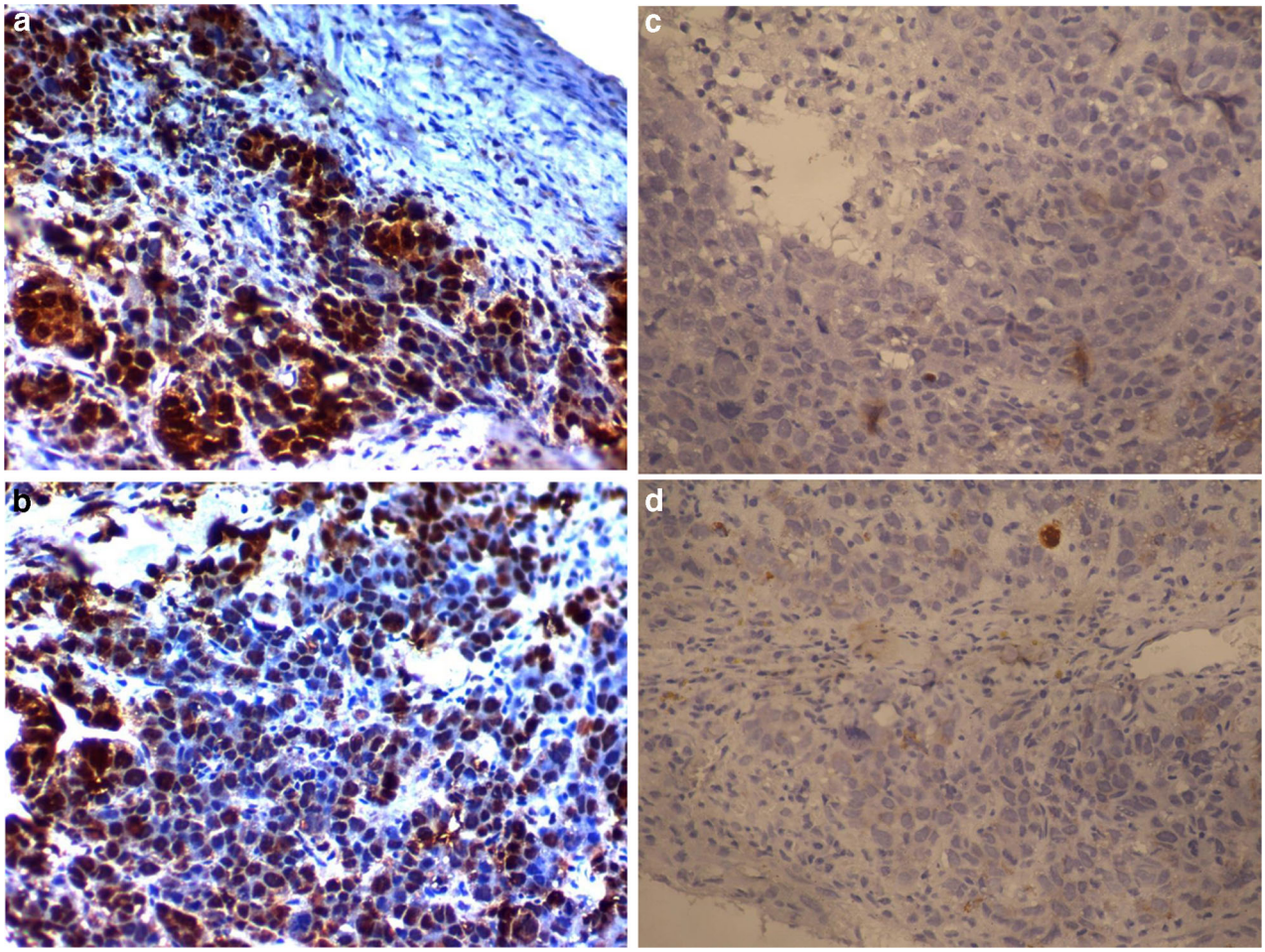

Fig. 4 Immunohistochemical expression of HIF-1 in HCC cells. **a** High expression in the nucleus and cytoplasm of HCC cells × 400. **b** High expression in the nucleus and cytoplasm of HCC cells × 400. **c** Low expression in HCC cells × 400. **d** Low expression in HCC cells × 400. **a**–**d** The original magnification was × 400

Table 7 Correlation between VEGF, HIF-1 tissue expression, and response to treatment of 30 HCC patients

Outcome	HCC ($N = 30$)		VEGF High expression ($N = 21$)		Low expression ($N = 9$)		p value
	No.	(%)	No.	(%)	No.	(%)	
Response to treatment							
PD	15	(50%)	15/15	(100%)	0/15	(0%)	< 0.001
SD	11	(36.6%)	6/11	(54.5%)	5/11	(45.5%)	
PR	4	(13.4%)	0/4	(0%)	4/4	(100%)	
PD	15	(50%)	15/15	(100%)	0/15	(0%)	< 0.001
OAR (PR + SD)	15	(50%)	6/15	(40%)	9/15	(60%)	
	HCC ($N = 30$)		High expression ($N = 18$)		Low expression ($N = 12$)		
	No.	(%)	No.	(%)	No.	(%)	
HIF-1							
Response to treatment							
PD	15	(50%)	12/15	(80%)	3/15	(20%)	< 0.001
SD	11	(36.6%)	6/11	(54.5%)	5/11	(45.5%)	
PR	4	(13.4%)	0/4	(0%)	4/4	(100%)	
PD	15	(50%)	12/15	(80%)	3/15	(20%)	< 0.001
OAR (PR + SD)	15	(50%)	6/15	(40%)	9/15	(60%)	

In a meta-analysis of 9 studies that evaluated the relationship between VEGF level and clinical outcome in advanced HCC patients treated with sorafenib, the pooled estimates suggested that high level of VEGF was associated with poor overall survival (HR = 1.85; 95% CI 1.24–2.77; $p = 0.003$) and poor progression-free survival (HR = 2.09; 95% CI 1.43–3.05; $p < 0.01$) in HCC, which was in agreement with our results [12, 14].

A lot of trials have examined the correlation between HIF-1 α and clinical outcome in HCC but the data is still conflicting [45–47]. A meta-analysis of total 7 studies, containing 953 HCC patients, showed that high HIF-1 expression associated with poor DFS and OS in HCC [24], and this was inconsistent with our results.

In Conclusion Combination of sorafenib with metformin did not have superior efficacy over sorafenib alone. The promising prognostic role of VEGF and HIF-1 α may allow their incorporation in the screening programs of HCC and to predict response to targeted therapy.

Compliance with Ethical Standards

This study was approved by Zagazig University Institutional Review Board (IRB), and carried out from December 2014 to January 2016 at Zagazig University and El Mabara Hospitals.

Conflict of Interest The authors declare that they have no conflicts of interest.

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