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Genes expression and serum biomarkers for diagnosis of hepatocellular carcinoma, cirrhosis and hepatitis C

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ABSTRACT – Background – Hepatocellular carcinoma (HCC) is the most common type of liver cancer. Risk factors for HCC include hepatitis C (HCV) and B (HBV) virus infection, alcoholic cirrhosis and genetic alterations that can affect several cellular pathways. **Objective –** This study purposed to analyze the gene and serum protein expression of vascular endothelial growth factor (VEGF), angiogenesis, alpha fetoprotein, cystatin B (CSTB), β-catenin and glypican-3 (GPC3) in groups with HCC, cirrhosis or HCV and controls, and their relation with clinical staging in the HCC and cirrhosis groups, as well its sensitivity and specificity values. **Methods –** A total of 230 individuals were distributed in Group 1 (G1) – 80 patients with HCC; Group 2 (G2) – 76 patients with cirrhosis due to any etiology; Group 3 (G3) – 33 patients with HCV; Group 4 (G4 – controls) – 41 individuals without clinical or biochemical signs of any liver disease. Gene expression was analyzed by qRT-PCR and serum proteins were performed using the ELISA method. **Results –** Increased VEGF and angiogenesis, alpha fetoprotein expression could be observed in BCLC stage-D patients compared to stage-B patients, and stage-C patients showed higher expression of β-catenin, compared to stage-B patients ($P<0.05$). For VEGF and GPC3, discriminatory power was observed between HCC patients and controls (AUC =0.71; 0.82, respectively). CSTB showed discriminatory power in the comparison between patients with HCV and controls (AUC =0.74). **Conclusion** – The present study confirms the sensitivity of serum CSTB in the diagnosis of hepatitis C, and gene expression of VEGF and serum GPC3, confer both sensitivity and specificity for the diagnosis of HCC.

Keywords – Hepatocellular carcinoma; angiogenesis; cell proliferation; signaling pathways.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the most frequent primary hepatic neoplasm of primary liver tumors and the main type of liver cancer, with more than half-million new cases diagnosed each year[1]. In the world, it is a leading cause of cancer-related death, with approximately 600,000 deaths per year[2].

Hepatocarcinogenesis results from a complex and heterogeneous malignant process, both molecularly and clinically, and occurs in the context of underlying liver dysfunction. As with other types of cancer, it is caused by alterations occurring in genomic DNA, with the combination of viral and environmental factors[3]. Molecular studies show the involvement of a multiple pathway process and the accumulation of genetic and epigenetic events, causing abnormal or inactivation of several signaling pathways, including cell proliferation, survival, differentiation and angiogenesis[4].

Genetic factors include vascular endothelial growth factor (VEGF) involved in angiogenesis, alpha fetoprotein (AFP) related to the promotion and development of tumors, as well as cystatin B (CSTB), β-catenin (CTNNB1) and glypican-3 (GPC3) related to protection, communication and cell signaling, respectively. The identification of biomarkers involved in HCC may contribute to the early diagnosis of the disease as well as to propose new therapeutic interventions. It should be emphasized that the combination of target therapies according to the genomic signatures of the tumors, characteristic of translational oncology, will eventually optimize the treatment and prognosis of patients[4,5].

Thus, the objectives of this study were to analyze gene expression of VEGF, AFP, CSTB, CTNNB1 and GPC3 in tumor tissue, as well as serum protein levels (VEGF, AFP and CSTB) in association with HCC and cirrhosis, and to determine the sensitivity and specificity of these serum proteins as potential diagnostic markers.

METHODS

A total of 230 individuals were distributed into Group 1 (G1) – 80 patients with HCC; Group 2 (G2) – 76 patients with cirrhosis due to any etiology; Group 3 (G3) – 33 patients with HCV; Group 4 (G4) – 41 individuals with no clinical or biochemical signs of any liver disease (controls). The clinical profile of all the patients

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studied was presented in TABLE 1. The patients were attended at the Gastroenterology Outpatient Clinic and at the Institute of Cancer (ICA) of the Base Hospital University Medical Center of the Medical School of São José do Rio Preto (HB-FAMERP), as well as at the Gastroenterology Service of the Hospital Center of the University of Coimbra, during 2015 and 2020. The diagnosis of HCC was performed according to the guidelines of the American Association for the Study of Liver Diseases (AASLD)[6]. The group with healthy controls (G4) was selected at the HB-FAMERP Blood Center and among patients submitted to surgery at the Gastroenterology Service of this institution, without any of these hepatopathies. Individuals with other neoplasms, diseases psychiatric or pregnant women were excluded. This study was approved by the Research Ethics Committee of the institution (CEP/FAMERP – Number 435/2014).

TABLE 1. Demographic profile, lifestyle, risk factors and clinical classification in patients with hepatocellular carcinoma (G1), cirrhosis (G2) and individuals without the disease (G3).

Variable	G1 (N=80)		G2 (N=76)		G3 (N=33)		G4 (N=41)	
Gender	N	(%)	N	(%)	N	(%)	N	(%)
Female	20	(25)	19	(25)	15	(45)	11	(25)
Male	60	(75)	57	(75)	18	(55)	30	(75)
Lifestyle								
Alcohol consumption	45	(56)	38	(50)	12	(36)	5	(13)
Smoking	40	(50)	26	(34)	16	(48)	9	(21)
Comorbidity								
HBV	17	(21)	8	(10)	0	(0)	0	(0)
HCV	40	(50)	37	(49)	33	(100)	0	(0)
Cirrhosis	67	(84)	76	(100)	0	(0)	0	(0)

Clinical Classification	G1 (N=80)	
BCLC	N	(%)
A	25	(31)
B/C	47	(59)
D	8	(10)

Hepatic tissue fragments were obtained by percutaneous trucut needle biopsy or after surgery (liver transplantation) and stored in cryogenic tubes with stabilization solution (RNA later® – life technologies). Total RNA was extracted with TRIzol® reagent (Ambion®) and quantified in QUBIT 2.0 fluorometer according to manufacturers' instructions. The cDNA strand was synthesized using the high capacity cDNA reverse transcription Kit (Applied Biosystems®, Foster City, CA, USA). Transcription levels were normalized by the GAPDH and β-actin genes and the qPCR reaction was conducted by the StepOnePlus Real Time PCR System (Applied Biosystems®). The relative gene expression of VEGF, CTNNB1, CSTB, GPC3 AND AFP was determined by the comparative method $2^{-\Delta\Delta Ct}$, which relates the mean expression level of the normalizing genes used as endogenous control and the mean expression level of the genes of interest for each sample[7]. These analyzes were performed in half of the groups studied with randomized selection. All samples were tested in triplicate and expressed as relative difference of n-times relative to the calibrator (Controls). Negative control was included for all reactions.

Serum dosages of VEGF, CSTB and GPC3 were performed by ELISA (Enzyme-Linked Immunosorbent Assay – Kit Cloud-Clone Corp®) on samples from all patients[8]. Protein concentrations were determined by comparing the optical density of the samples with the standard curve. Serum levels of AFP were obtained from patients' digital records patients with HCC and cirrhosis, submitted to the analysis of gene expression (N=47), were also clinically classified according to the evolution of the disease, using the criteria of Barcelona Clinic Liver Cancer (BCLC) staging system or Child-Pugh.

Quantitative variables with Gaussian distribution were submitted to analysis of variance (ANOVA) for the comparison of three or more groups, and the *t*-test for two groups. For the non-parametric quantitative variables, the Kruskal Wallis test was applied in the comparison among three or more groups, and Mann Whitney, for two groups. Spearman's rank correlation coefficient analysis was performed between the expression levels of VEGF, CSTB, AFP, GPC3 and CTNNB1, and also for serum levels of VEGF, CSTB, AFP and GPC3. To determine values of sensitivity, specificity, positive predictive and negative predictive, the receiver operating characteristic (ROC) curve was used, setting areas under the curve ≥0.7 as clinical relevance. A box-plot graphical representation was used including minimum value, interquartile range, median and maximum values, as well as outliers. Alpha error was set at 5%. The Minitab, Stats Direct and GraphPad programs were used in the analyses.

RESULTS

Overexpression of VEGF in G1 (median =3.85) was observed, compared to G2 (0.76) and G3 (1.95; *P*=0.024) (FIGURE 1A). There were similarities in the levels of CTNNB1 expression between the groups (HCC: 1.97; cirrhosis: 1.86 and HCV: 1.21; *P*=0.775; FIGURE 1B). For GPC3 and AFP, increased values were observed in patients with HCC (4.47 and 3.60, respectively), compared to patients with cirrhosis (3.50 and 2.65, respectively) and hepatitis C (2.65 and 0.62, respectively), however, with no significant differences (*P*>0.05; FIGURE 1C-D). G3 also showed an increase in CSTB expression when compared to G1 and G2, although with no significant differences between them (7.49 versus 4.14 and 4.89, *P*> 0.05, for both; FIGURE 1E).

The respective gene expressions were related to the HCC staging system, with high levels of VEGF and AFP expression in BCLC stage-D patients (19.97 and 8.86, respectively; TABLE 2), compared to stage-B patients (1.96 and 0.03; *P*=0.008 and *P*=0.001). Stage-C patients showed higher CTNNB1 expression, compared to stage-B patients (18.40 versus 0.90; *P*=0.008). There were similarities between the expression levels of the other genes for the clinical classification of HCC patients (*P*>0.05). The same occurred for patients with cirrhosis classified by Child-Turcotte-Pugh score (*P*> 0.05).

In relation to serum protein levels, represented by median and quartiles values, there was an increase of GPC3 in the groups with HCC and cirrhosis (2.8 ng/mL and 2.9 ng/mL, respectively), compared to the controls (0.84 ng/mL, *P*=0.001 for both). On the other hand, values in the group with patients with HCV (2.0 ng/mL) were similar to the controls (*P*>0.05, FIGURE 2C). For CSTB, increased serum levels were observed in patients with HCC

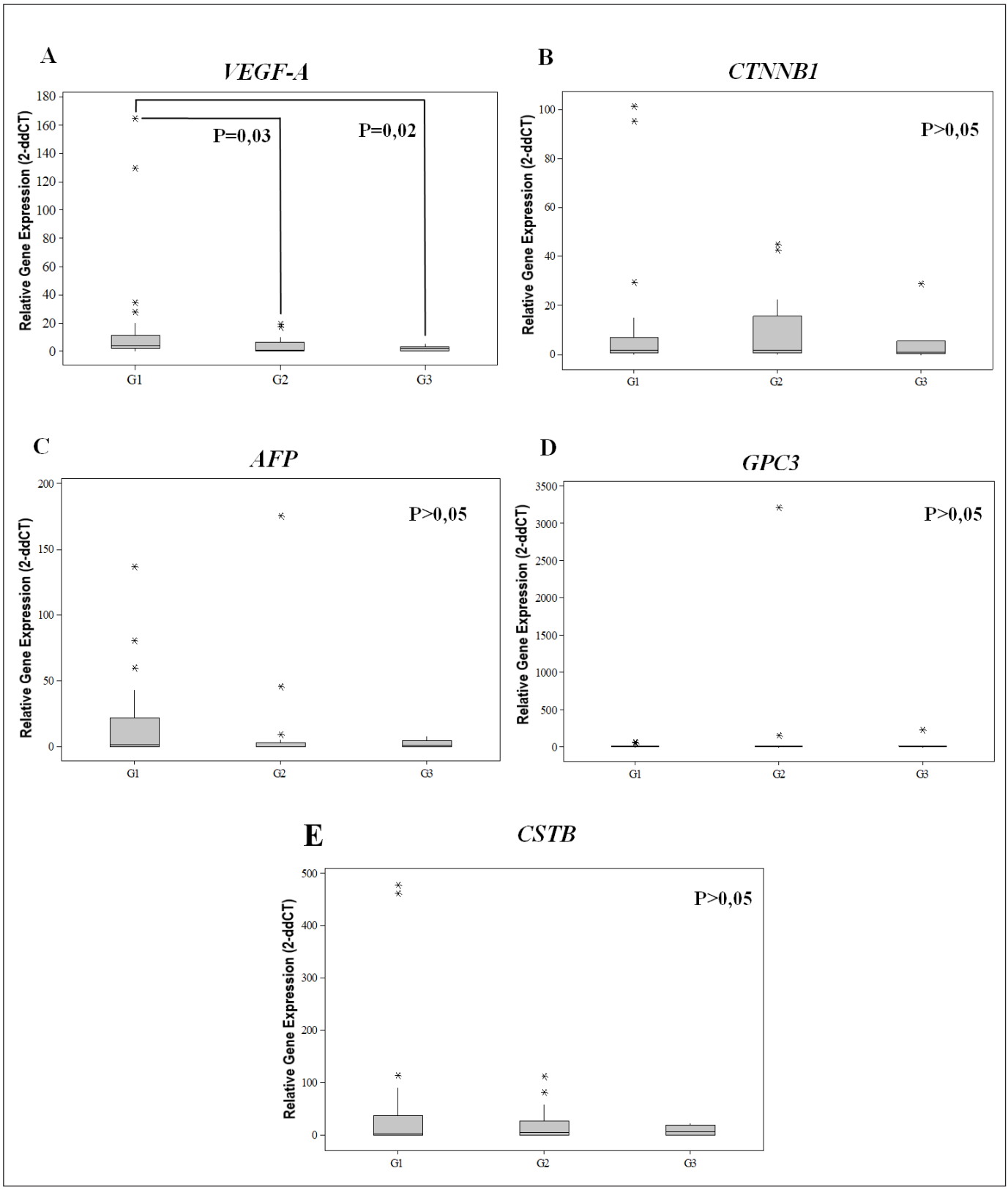

FIGURE 1. Schematic representation by "box-plot" of median values and gene expression quartiles of: **A)** vascular endothelial growth factor (VEGF); **B)** β-catenin (CTNNB1); **C)** alpha-fetoprotein (AFP); **D)** glypican-3 (GPC3) and E) cystatin-B (CSTB).

TABLE 2. Levels of gene expression for VEGF, AFP, CTNNB1, GPC3 and CSTB in patients with hepatocellular carcinoma, classified according to criteria of the BCLC group.

Stage	VEGF	AFP	CTNNB1	GPC3	CSTB
A (N=7)					
Median	2.58	1.82	0.54	5.00	0.39
Minimum	0.03	0.0001	0.0003	0.19	0.0001
Maximum	10.73	137.40	6.97	56.25	48.05
B (N=6)					
Median	1.96*	0.03*	0.90*	0.58	9.53
Minimum	0.15	0.0001	0.01	0.0007	0.25
Maximum	164.60	0.26	1.30	7.11	51.90
C (N=6)					
Median	4.89	4.10	18.40*	12.27	16.49
Minimum	2.01	0.03	1.20	0.40	0.35
Maximum	164.60	43.01	101.25	38.95	460.49
D (N=7)					
Median	19.97*	8.86*	3.96	25.06	11.20
Minimum	1.40	0.38	0.54	0.66	0.16
Maximum	130.01	80.64	14.98	68.59	477.15
*P-value	0.008	0.001	0.008	0.074	0.363

VEGF: vascular endothelial growth factor; AFP: alpha-fetoprotein; CTNNB1: β-catenin; GPC3: glipican-3; CSTB: cystatin-B; BCLC Barcelona Clinic Liver Cancer. *Mann-Whitney Test. The Kruskal-Wallis was used for the other with $P>0.05$.

(0.99 ng/mL) and HCV (1.0 ng/mL), compared to controls (0.6 ng/mL; $P=0.002$; $P=0.008$, respectively; FIGURE 2D). Patients with HCC also showed increased levels of VEGF (264.8 pg/mL) compared to the group with cirrhosis (185.2 pg/mL; $P=0.0007$), HCV (188.8 pg/mL; $P=0.03$) and controls (182.2 pg/mL; $P=0.009$; FIGURE 2A). For AFP, increased values were observed in the group with HCC (20.3 ng/mL), compared to the group with cirrhosis (2.6 ng/mL) and with HCV (3.0 ng/mL; $P<0.001$, for both; FIGURE 2B).

The ROC curve, considering serum protein levels, was used to evaluate the discriminative power of each variable (FIGURE 3). GPC3 was relevant to differentiate patients with HCC (G1) from controls (G4), with sensitivity of 82% and specificity of 72.5% (area under the curve =0.82), positive and negative predictive value of 0.80 and 0.74, respectively, and a cut-off value of 1.56 ng/mL. The same occurred in patients with cirrhosis versus controls, with specificity of 87% and sensitivity of 55% (area under the curve =0.75), positive and negative predictive value of 0.83 and 0.62, respectively, and cut-off value of 2.72 ng/mL, as well as between patients with HCV and controls, with sensitivity of 78% and specificity of 65% (area under the curve =0.72), positive and negative predictive value of 0.56 and 0.83, respectively, and cut-off value of 1.20 ng/mL.

Regarding serum VEGF levels, discriminatory power was observed between patients with HCC and controls, with sensitivity of 79% and specificity of 57% (area under the curve =0.71), positive and negative predictive value of 0.781 and 0.56 respectively, and

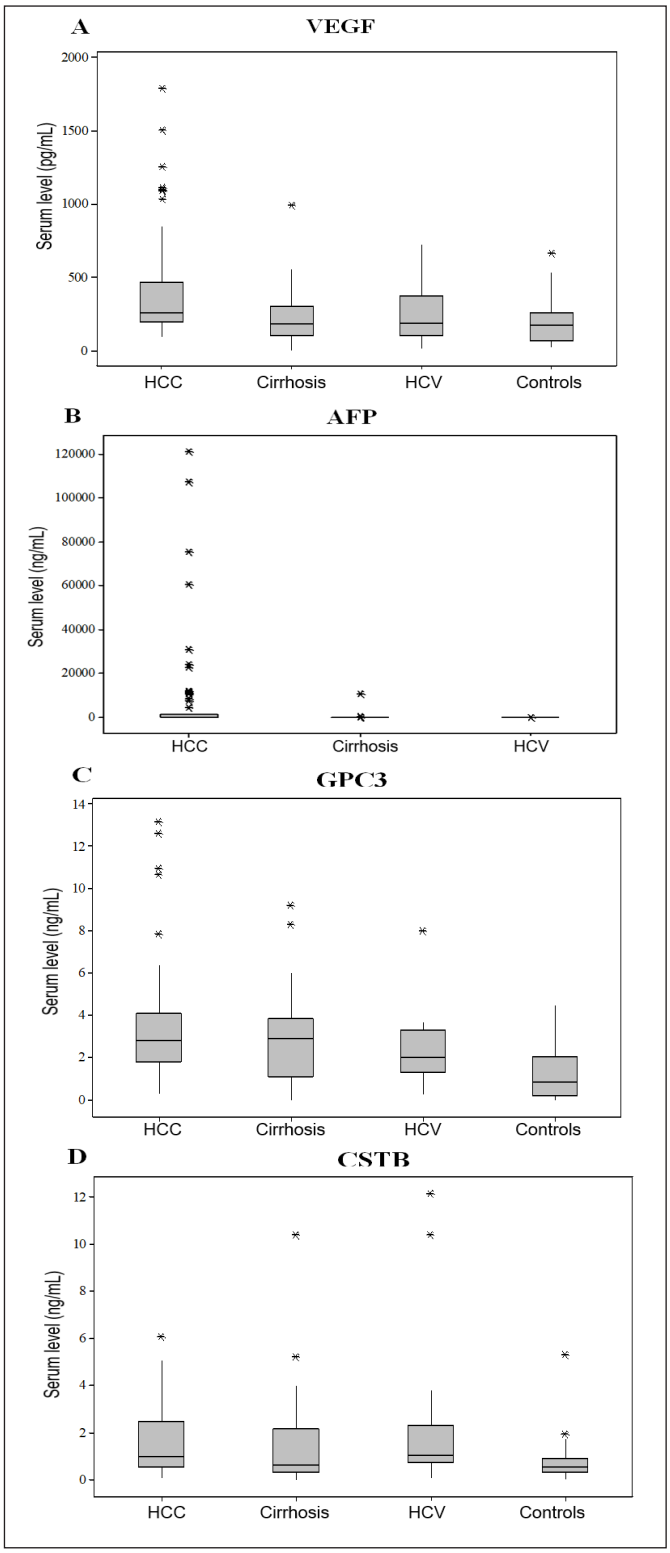

FIGURE 2. Schematic representation by "box-plot" of median values and quartiles of serum levels of **A)** Vascular endothelial growth factor (VEGF); **B)** alpha-fetoprotein (AFP); **C)** glypican-3 (GPC3) and **D)** cystatin-B (CSTB) in patients with hepatocellular carcinoma (HCC), cirrhosis, hepatitis C (HCV) and controls.

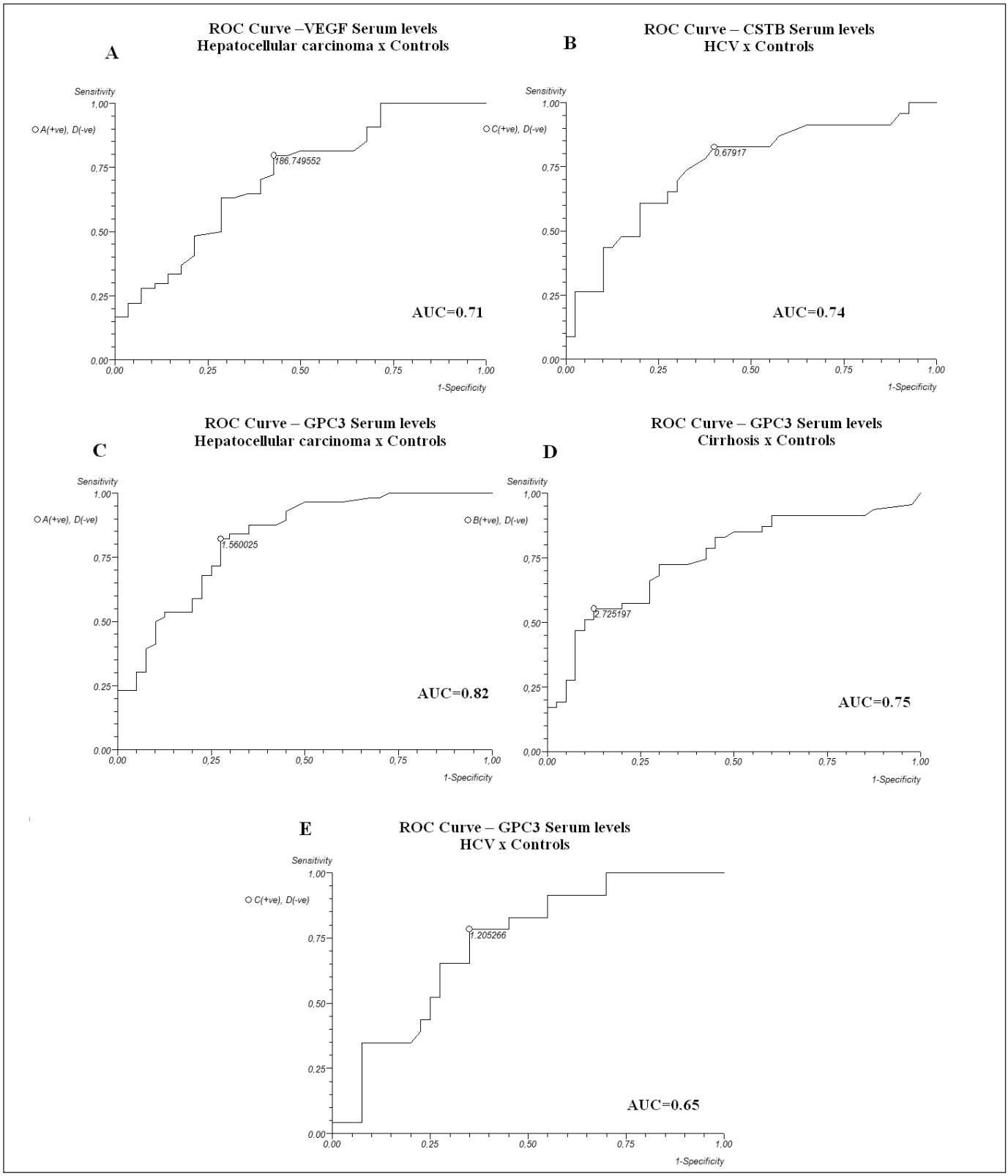

FIGURE 3. Receiver operator characteristic (ROC) curve of serum levels for: **A)** vascular endothelial growth factor (VEGF) between patients with hepatocellular carcinoma (HCC; G1) and controls (G4); **B)** cystatin-B (CSTB) between patients with hepatitis C (G3) and controls (G4); glypican-3 (GPC3) among patients: **C)** with HCC and controls; **D)** cirrhosis and controls; **E)** hepatitis C and controls.

cut-off value of 186.7 pg/mL. Also, discriminatory power for CSTB was observed in the comparison between the groups patients with HCV and controls, with sensitivity of 82% and specificity of 60% (area under the curve =0.74), positive and negative predictive value of 0.54 and 0.85, respectively, and cut-off value of 0.679 ng/mL).

For gene expression levels in G1, the correlation analysis (TABLE 3) showed positive correlation between all genes except CSTB expression, correlated only with CTNNB1 (r=0.43; *P*=0.01), and GPC3 with VEGF (r=0.49; *P*=0.008). For the other groups, the correlation between CTNNB1 and GPC3 can be pointed out (r=0.52; *P*=0.004). In G2, there was a positive correlation between expression levels of all genes, with emphasis on GPC3 and VEGF (r=0.94; *P*<0.0001), followed by CTNNB1 with VEGF and GPC3 (r=0.88; r=0.87; *P*<0.0001, for both). For G3, there was a strong positive correlation between all genes, except for CSTB related only with AFP (r=0.85; *P*=0.01), and with CTNNB1 (r=0.85; *P*=0.01). For the other groups, the correlation between GPC3 and VEGF (r=1.00; *P*=0.0004) was observed, followed by VEGF with AFP and CTNNB1 (r=0.91; r=0.90; *P*=0.001; *P*=0.004, respectively).

DISCUSSION

In this study, the expression analysis of genes related to angiogenesis VEGF, tumors promotion and development AFP, protection CSTB, communication β-catenin and cell signaling GPC3 showed high values only of VEGF in hepatic tumor tissue of HCC patients, supporting the other studies results[8,9]. Overexpression of VEGF in HCC patients is due to tumor hypervascularization, since solid tumors require the blood supply for the nutrients transport, oxygen and waste removal for cell viability and proliferation. In this context, to generate blood supply, several malignancies increase the expression of VEGF and its receptors, becoming hypervascularized, and consequently more invasive and metastatic[9,10]. The association of VEGF overexpression with HCC helps produce anti-angiogenic drugs used in the cancer treatment. VEGF and its protein can be pointed out as the major therapeutic targets for the treatment of neoplasias[11,12].

Higher serum levels of VEGF were relevant in discriminating HCC patients from controls, with values of 79% sensitivity and 57% specificity, suggesting their potential role in the HCC diagnosis, as already demonstrated for bladder cancer[13]. In a previous study, our research group found a relationship between increased VEGF and VEGF-C936T polymorphism in HCC patients[14,15].

Although GPC3 has similar gene expression among the groups, increased serum levels of serum glypican-3 were observed in HCC patients, adding high sensitivity and specificity (82% and 72.5%, respectively), with diagnostic potential for the disease. These findings agrees other studies[16,17]. Notably, GPC3 is a protein anchored to the plasma membrane and is involved in cell growth, differentiation and migration due to the regulation of grow factor signaling, including fibroblast growth factors, Hedgehog proteins and WNT pathways[17,18].

In this context, serum changes of GPC3 in HCC may influence tumorigenesis[19]. Although GPC3 is absent in hepatocytes of healthy individuals and patients with non-cancerous liver disease, it can be detected in about 50% of HCC patients and 33% of those with the disease, but with normal AFP values[20]. Lee et al. (2014) also reported increased serum levels of GPC3[21]; however, with less expressive values of sensitivity and specificity (53.8% and 65%, respectively). There is also reference to GPC3 overexpression in HCC patients[15], and similarity between serum levels of individuals with and without HCC[20]. In this case, the group with healthy controls was composed of individuals with chronic liver diseases, which possibly prevented differentiation between patients and controls.

In the present study, unlike Hass et al. study (2015), there were similarities between the groups in relation to the AFP gene

TABLE 3. Correlation between gene expression of VEGF, AFP, CTNNB1, GPC3 and CSTB in patients with HCC, cirrhosis and hepatitis C.

GENE	Group	*AFP*		*GPC3*		*CTNNB1*		CSTB	
		(r)	*P*-value	(r)	*P*-value	(r)	*P*-value	(r)	*P*-value
VEGF	HCC	0.38	0.04	0.48	0.008	0.47	0.01	0.21	0.26
	Cirrhosis	0.53	0.03	0.94	0.0001	0.88	0.0001	0.70	0.002
	Hepatitis C	0.91	0.001	1.00	0.0004	0.90	0.004	0.69	0.06
AFP	HCC			0.14	0.94	0.39	0.03	0.01	0.94
	Cirrhosis			0.52	0.04	0.57	0.02	0.52	0.03
	Hepatitis C			0.89	0.01	1.00	0.0001	0.85	0.01
GPC3	HCC					0.52	0.004	0.12	0.51
	Cirrhosis					0.87	0.0001	0.61	0.01
	Hepatitis C					0.82	0.05	0.60	0.24
CTNNB1	HCC							0.43	0.01
	Cirrhosis							0.86	0.0001
	Hepatitis C							0.85	0.01

VEGF: vascular endothelial growth factor; AFP: alpha-fetoprotein; CTNNB1: β-catenin; GPC3: glipican-3; CSTB: cystatin-B; HCC: hepatocellular carcinoma. *Spearman rank correlation (r).

expression[17]. However, HCC patients at stage D showed AFP overexpression, compared to patients at stage B, considered to have a better prognosis[22]. The BCLC staging system considers number, size of tumors and the performance status and Child-Pugh score[23]. Patients in the final stage of the disease (BCLC-D) present with severe hepatic lesions, whose clinical characteristics are associated with elevated AFP, including a larger number of tumors or larger in diameters and deficiency in liver function due to chronic histological damage. Additionally, patients with early-stage HCC (BCLC-A) showed increasing levels of AFP mRNA. It is noteworthy that serum levels of AFP may increase in the early stages of HCC and then decline or normalize at disease progression, making AFP the most commonly used tumor marker for HCC[24]. Nevertheless, a recent study challenges the AFP diagnostic power[25].

Although the CSTB gene expression showed similarity between the groups, there is reference of tumor up-regulation in HCC patients[26]. It is worth mentioning that in our, the HCV patients group showed increasing expression of CSTB compared to the group with HCC and cirrhosis. On the other hand, in our study, using a cut-off level of 0.68 ng/mL, the CSTB serum quantification proved to be a potential tool to discriminate HCV patients from healthy individuals, with sensitivity of 82% and specificity of 60%.

Cystatins, endogenous inhibitors of cysteine proteases such as L, H and S cathepsins regulate neutrophil chemotaxis, tissue inflammation and resistance to bacterial and viral infections[27,28]. Thus, these results reinforce the gene role in coding proteins to protect against viral infection, such as the hepatitis C virus infection. Differently, in the present study, the CSTB serum levels increase in HCC patients showed sensitivity of 53% and specificity of 80% in the distinction between patients and controls, in agreement with another study. Importantly, higher serum levels of CSTB have also been reported in ovarian cancer[29].

Studies involving the gene expression of CTNNB1 and HCC are scarce in the literature, which makes it difficult to discuss the data presented here. On the other hand, there is reference of high protein expression in HCC patients tumor tissue[30]. In fact, when WNT binds to the Frizzled receptor and the LPR5/6 co-receptor, glycogen synthase kinase 3 is recruited into the cell membrane thereby inhibiting its activity and increasing ß-catenin activity. Next, β-catenin migrates to the nucleus of cells to perform various functions, such as abnormal cell growth and possible carcinogenesis[30-32].

In this study, VEGF gene expression correlated positively with all genes studied, indicating the relationship of the angiogenesis process with genes involved in proliferation, communication and cell signaling pathways. The WNT pathway regulates the transcription of genes responsible for these processes. In the absence of this ligand, β-catenin is degraded and is not accumulated in the cytoplasm[31]. Differently, for the GPC3 gene, which regulates the signaling activity of several growth factors, there is autocrine/paracrine regulation of WNT signaling considering its effect on the HCC cells growth[33].

It is well-established that the advances in molecular biology have enabled researchers to track genes and proteins involved in hepatocarcinogenesis[34,35], consequently influencing the diagnosis and prognosis of the disease. Thus, the present study confirms the sensitivity of serum CSTB in the hepatitis C diagnosis (related to angiogenesis and cell proliferation), represented by the gene and protein overexpression of VEGF and GPC3 serum, respectively, add both sensitivity and specificity for the HCC diagnosis. This work represents an advance in biomedical science because it shows the sensitivity of serum CSTB in the diagnosis of hepatitis C, and that gene expression of VEGF and serum GPC3, confer both sensitivity and specificity for the diagnosis of HCC.

Authors' contribution

Fernandes-Ferreira R: study design, data collection, statistical analysis and scientific writing; Tenani GD: data collection; Pinhel MAS: statistical analysis and scientific writing; Abrantes AMC: data collection and article text review; Botelho MFRR: study design and data collection; Silva RCMA: study design and data collection; Souza DRS: study design and article text review; Silva RF: study supervisor and article text review.

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Fernandes-Ferreira R, Tenani GD, Pinhel MAS, Abrantes AMC, Botelho MFRR, Silva RCMA, Souza DRS, Silva RF. Expressão gênica e biomarcadores séricos para diagnóstico de carcinoma hepatocelular, cirrose e hepatite C. Arq Gastroenterol. 2022;59(3):394-401.

RESUMO – Contexto – Carcinoma hepatocelular (CHC) é o tipo mais comum de câncer de fígado. Os fatores de risco para CHC incluem infecção pelo vírus da hepatite C (VHC) e B (VHB), cirrose alcoólica e alterações genéticas que podem afetar diversas vias celulares. **Objetivo –** Este estudo teve como objetivo analisar a expressão gênica e proteica sérica de VEGF, AFP, CSTB, β-catenina e GPC3 em grupos com CHC, cirrose ou VHC e controles, e sua relação com o estadiamento clínico nos grupos CHC e cirrose, bem como sua valores de sensibilidade e especificidade. **Métodos –** Duzentos e trinta indivíduos foram distribuídos no Grupo 1 (G1) – 80 pacientes com CHC; Grupo 2 (G2) – 76 pacientes com cirrose de qualquer etiologia; Grupo 3 (G3) – 33 pacientes com VHC; Grupo 4 (G4 – Controles) - 41 indivíduos sem sinais clínicos ou bioquímicos de qualquer doença hepática. A expressão gênica foi analisada por qRT-PCR e as proteínas séricas foram realizadas pelo método ELISA. **Resultados –** Aumento da expressão de VEGF e AFP pode ser observado em pacientes BCLC estágio D em comparação com pacientes estágio B, e pacientes estágio C apresentaram maior expressão de CTNNB1, em comparação com pacientes estágio B (P<0,05). Para VEGF e GPC3, foi observado poder discriminatório entre pacientes com CHC e controles (AUC = 0,71; 0,82, respectivamente). O CSTB mostrou poder discriminatório na comparação entre pacientes com VHC e controles (AUC =0,74). **Conclusão –** O presente estudo confirma a sensibilidade do CSTB sérico no diagnóstico da hepatite C, e a expressão gênica de VEGF e GPC3 sérica conferem sensibilidade e especificidade para o diagnóstico de CHC.

Palavras-chave – Carcinoma hepatocelular; angiogênese; proliferação celular; vias de sinalização.

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