Research Article
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Circulating D-dimer level may predict tumor burden in chronic hepatitis C related hepatocellular carcinoma

Abstract

Introduction: Coagulation and fibrinolysis activation has been implicated in angiogenesis, tumor cell invasion, tumor progression and metastatic spread. D-dimer is a biomarker that globally indicates the activation of hemostasis and fibrinolysis.

Aim: To study the correlation of circulating D-dimer level with the tumor grade in HCV related HCC.

Methods: HCC was diagnosed by non- invasive method in cirrhotic patients according to EASL criteria using contrast enhanced abdominal CT and or MRI. After metastatic work up, HCC grade was described as grade one if it was within Milan criteria (one nodule <5cm or up to 3 nodules; the largest is <3cm without vascular invasion or extra- hepatic involvement); grade two if it was beyond Milan criteria but non –metastatic & grade three if it was metastatic (vascular invasion, lymph node metastasis, distant metastasis). BCLC staging depending on HCC burden, Child -Turcotte-Pugh (CTP) class and ECOG performance status (PST), was done for all studied cases. Chromic HCV was diagnosed by 4th generation ELIZA for HCV antibody and confirmed by quantitative PCR for HCV RNA. Circulating D dimer level, serum AFP level, liver biochemical tests, serum creatinine & complete blood count were measured for all studied cases. Patients with history of previous treatment for HCC; recent benign thrombo-embolic disorder or renal impairment were excluded.

Results: Fifty chronic HCV related HCC cases; 43 males and 7 females with age 62.2±7.4 years were enrolled. Circulating D-dimer level was significantly higher in HCC grade 2 and 3 than HCC grade 1 [(400-800) versus (0-400 ng/ml), p=0.001] & in BCLC stage D than BCLC stage C HCC [(400-800) versus (0-700 ng/ml), p=0.021].There was significant positive correlation between circulating D-dimer level and HCC size and HCC grade (r =0.35, p = 0.012, r =0.33, p = 0.016 respectively). There was also significant positive correlation between circulating D-dimer level and both serum AFP level (r =0.45, p=0.001) and ECOG performance status (r =0.4, p = 0.002). Serum level of circulating D-dimer at cut off level ≥300 ng/ml can predict HCC grade beyond Milan criteria (G2& 3) with PPV of 77.1%, NPV of 73.3%, AUC= 0.8 and p <0.0005.Also, serum AFP level at cut off ≥394.5 can predict HCC grades beyond Milan criteria with PPV of 89.3%, NPV of 72.7%, AUC= 0.81 and P<0.0005.

Conclusion: In HCV related HCC, circulating D-dimer level was positively correlated with HCC grade and largest tumor size with higher level in HCC grades 2&3 as well as BCLC stage D. Cut off level of circulating D-dimer level of ≥300 ng/ml can predict HCC grades beyond Milan criteria.

Keywords: hepatocellular carcinoma, chronic hepatitis C, D- dimer, alpha fetoprotein, milan criteria, liver transplantation, Egypt

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Abbreviations: AFP, alpha fetoprotein; AFP-L3, lens culinaris agglutination reactive AFP; AFU, alpha-l-fucosidase; AIH, auto immune hepatitis; ALT, alanine amino transferase; ANOVA, analysis of variance; AST, aspartate amino transferase; AUC, area under curve; BCLC, barcelona clinic liver cancer; CBC, complete blood count; CI, confidence interval; CRP, c-reactive protein; CT, computed tomography; CTP, child-turcotte-pugh; DCP, des-gamma carboxy prothrombin; DM, diabetes milletus; DVT, deep vein thrombosis; EASL, european association for the study of liver; ECOG, eastern co-operative oncology group; ELISA, enzyme-linked immuno sorbent assay; ESR, erythrocyte sedimentation rate; FDA, food and drug administration; FDPs, fibrin degradation products; HBV, hepatitis b virus; HCC, hepato cellular carcinoma; HCV, hepatitis c virus; INR, international normalized ratio; MDCT, multi detector CT; MRI, magnetic resonance imaging; MWA, microwave ablation; NASH, non-alcoholic steatohepatitis; NPV, negative predictive value; OR, odds ratio; OS, overall survival; PEI, percutaneous ethanol injection; PPV, positive predictive value; PST, patient status of performance; PT, prothrombin time; PVT, portal vein thrombosis; RBCs, red blood cells; RFA, radiofrequency ablation; ROC, receiver operating characteristic curve; SD, standard deviation; SE, standard error; SMH, specialized medical hospital; Sn, sensitivity; SOR, sorafenib; Sp, specificity; TACE, trans-arterial chemoembolization; TNM, tumor, nodes, metastases; TPA, tissue plasminogen activator; US, ultra sound; VEGF, vascular endothelial growth factor; VTE, venous thrombo embolism; WBCs, white blood cells

Introduction

Chronic HCV infection causes a chronic hepatitis process leading to liver fibrosis, cirrhosis, hepatocellular carcinoma (HCC) and death.[1] HCC pathogenesis on molecular level is remarkably complex and can vary considerably within tumor.[2,3] Despite remarkable advance in diagnosis and therapy, long term survival rates remain poor.[4,5] Many pathological conditions lead to activation of coagulation and fibrinolysis.[6,7] Coagulation and fibrinolysis markers have the potential to serve as predictors of disease severity. An increasing body of evidence suggests a relationship between activation of coagulation and tumor aggressiveness.[8,9,10,11] D-dimer is degradation product of the cross linked fibrin that is degraded by plasmin during fibrinolysis and it is therefore a measure of both fibrinolysis and coagulation.[12,13] Previous studies have reported that circulating D-dimer level was a predictor of poor prognosis in several types of malignancies including ovarian,[10] breast,[14] colorectal,[15] lung,[16,17] gastric[18] and pancreatic cancer.[19] In lung cancer, circulating D-dimer level has been shown to be associated with tumor stage, response to treatment and survival.[11,20,21] In ovarian cancer, pre-treatment circulating D-dimer level was a predictor of disease progression and outcome.[10] Recently, pre-treatment circulating D-dimer level was also reported to be an effective predictor of prognosis in T cells in lymphoma.[23]

Aim of the study

To study the correlation of circulating D-dimer level with the tumor grade in HCV related HCC.

Methods

This was a cross sectional study which included 50 naive HCV related HCC adult patients who were admitted to inpatient wards of Gastroenterology and Hepatology Unit of Specialized Medical Hospital (SMH) in Mansoura University in Egypt. Exclusion criteria were the presence of arterial or venous thromboembolism in the preceding 3 months; anticoagulant therapy within preceding 6 months; known congenital coagulation abnormality; previous treatment for HCC either surgical or non-surgical (86 cases); HCC related to other causes of liver diseases (4 cases); focal hepatic lesions other than HCC (12 cases) and presence of chronic renal impairment (one case) (Figure 1). The study duration extended from August 2017 to July 2018. All enrolled patients were subjected to detailed medical history, clinical evaluation, assessment of performance status (PST) by scale of the Eastern Cooperative Oncology Group (ECOG) (Table 1).

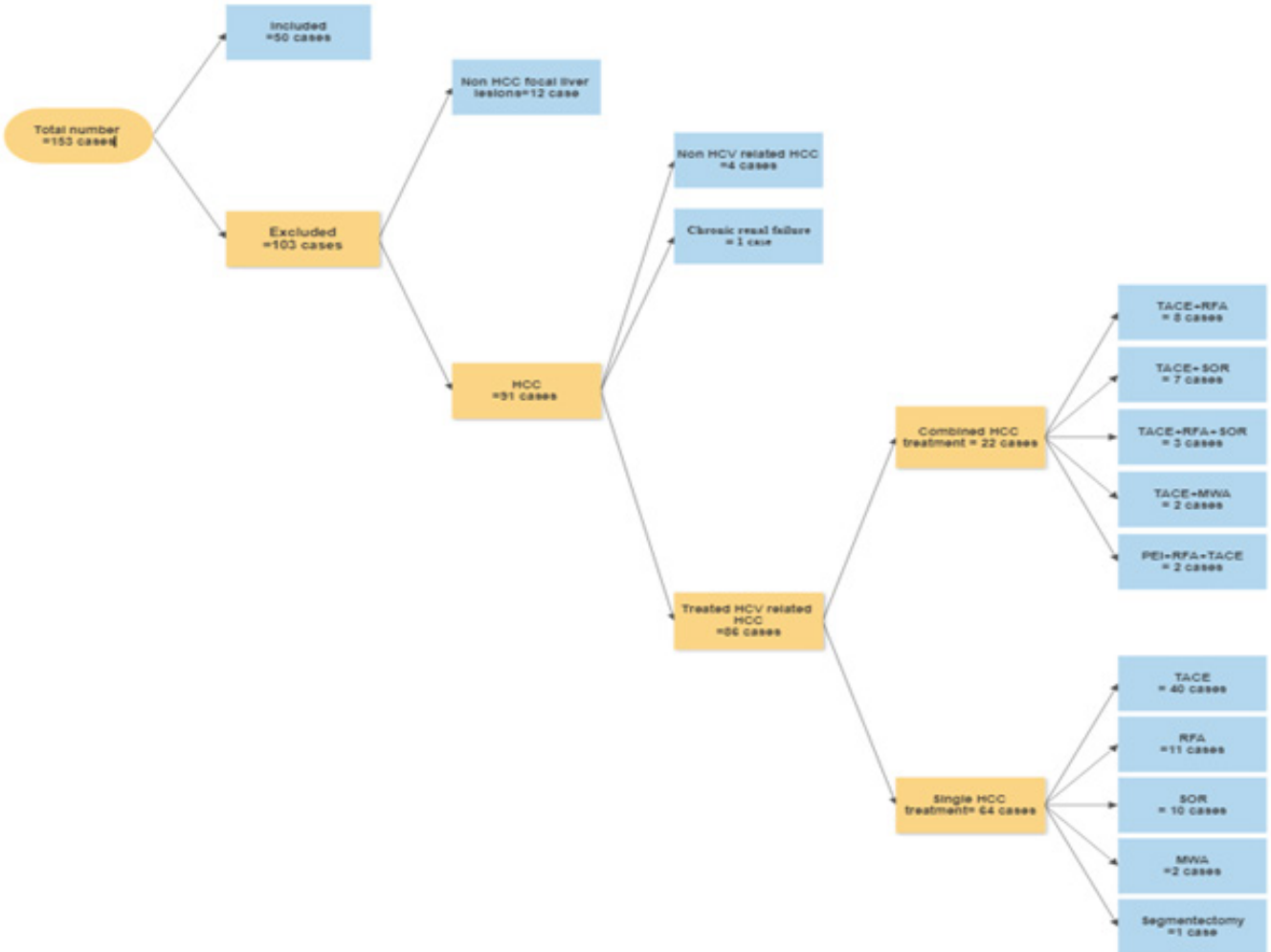

Figure 1 Flowchart of the studied cases.

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Table 1 Assessment of patient performance status (PST) by scale of the Eastern Cooperative Oncology Group (ECOG)[23]

Grade	ECOG performance status
0	Fully active, No restriction of pre-disease performance
1	Restriction of physically strenuous activity only
2	Unable to carry out any work activities, but ambulatory and capable of all self-care
3	Confined to bed or chair more than 50% of time, Capable of only limited self-care.
4	Totally confined to bed or chair, cannot carry on any self-care
5	Dead

Laboratory testing of the patients included serum albumin (gm/dl), bilirubin (mg/dl), aspartate amino transferase (AST) (u/l), alanine amino transferase (ALT) (u/l), prothrombin time in seconds (PT), International normalized ratio (INR), serum creatinine (mg/dl), Complete blood count (CBC), 4th generation ELISA for HCV antibody, quantitative PCR for HCV RNA, serum alpha fetoprotein level (ng/ml) & circulating D-dimer level (ng/ml). Circulating D-dimer level was measured using patient plasma which was prepared from whole blood and anti-coagulated with sodium citrate. Agglutination assay was done using latex beads with a highly specific D-dimer monoclonal antibody. Radiological investigations included abdominal ultrasound, muliphasic contrast enhanced CT (MPCT) and or Dynamic contrast enhanced MRI of the abdomen for HCC non-invasive diagnosis in cirrhotic patients according to EASL criteria. Metastatic workup including CT brain, chest and pelvis & bone scan were done. HCC grade was described as grade one if it was within Milan criteria (one nodule <5 cm or up to 3 nodules; the largest is <3 cm without vascular invasion or extra- hepatic involvement); grade two if it was beyond Milan criteria but non–metastatic & grade three if it was metastatic (vascular invasion, lymph node metastasis, distant metastasis). BCLC staging depending on HCC burden, Child-Turcotte -Pugh (CTP) class and ECOG performance status (PST), was done for all studied cases.

Ethical approval

The study protocol was reviewed and approved by the ethical committee at Faculty of Medicine in Mansoura University; the approval code was MS/17.05.154. Written informed consents were obtained from the patients after assuring confidentiality.

Sample size

Sample size was calculated by PASS software version 2008. Sample size estimation depended on a study of Lui et al.[24] In their study, elevation of both fibrinogen and D-dimer were significantly correlated with both largest and total HCC tumor size (p=0.001). However, r value was unfortunately not mentioned by the authors. A sample size of 50 patients achieves 95% power to detect a difference between the null hypothesis and the alternative hypothesis with a significant level of 0.05.

Statistical analysis

Data were entered and analyzed using IBM-SPSS software version 26. Quantitative data were expressed as mean ± standard deviation (SD) if normally distributed .Qualitative data were expressed as frequency (N) and percentage (%). Qualitative data were compared by Chi-Square test or Fisher's exact test. Quantitative data were compared by Independent-Samples t-Test or Mann-Whitney U test. The Spearman's rank-order correlation was used to assess the strength and direction of the association between two continuous variables or between one continuous variable and one ordinal variable. The univariate analyses and multivariate logistic regression analyses were conducted to determine predictors of HCC grade. ROC curve was used for estimation of cut off level of circulating D dimer level in prediction of HCC grade. Results were considered statistically significant if p value was ≤ 0.05.

Results

Table 2 shows that fifty chronic HCV related HCC cases with age 62.2±7.4 years were enrolled. Male to female ratio was 6:1. 40% of the patients were smokers; 52% were rural and 48% were urban. The most common presenting symptoms were abdominal pain and abdominal mass (respectively). History of upper gastrointestinal bleeding due to ruptured varices was present in 16% of the cases. Diabetes Mellitus (DM) was present in 28% and hypertension was present in 16% of the cases. The PST of the enrolled patients was grade 1 in 28%; grade 2 in 54%; grade 3 in 10% and grade 4 in 8% of the cases. HCC was uni-focal in 66 %; ≤ 3 focal lesions in 22% and >3 focal lesions in 12% of the cases. The diameter of largest tumor was <3cm in 6% and ≥ 3 cm in 94% of cases. Vascular invasion was present in 20% and distant metastasis was present in 2% of cases. As regards CTP class, 8% had CTP class A; 32% had CTP class B and 60% had CTP class C. BCLC staging showed that 40% of HCC cases had stage C while 60% had stage D. HCC grading showed that 38% of the cases were within Milan while 62% of them were beyond Milan criteria. Serum level of AFP was < 200 ng/ml in 4% and ≥ 200 ng /ml in 96% of cases.

Table 2 Characteristics of the enrolled HCC patients

Variable	Value (n, %)
Age (years) (mean ± SD)	62.2±7.4 years
Gender (male/ female)	43/7(86 /14%)
Residence (urban/rural)	/26(48 /52%)24
Current smoking	20 (40%)
Presenting symptom: Pain/ mass/both/no	40/2/4/4 (80 /4 /8 /8%)
Bleeding varices	8 (16%)
Diabetes Mellitus	14(28%)
Hypertension	8(16%)
PST: grade1/2/3/4	14/27/5/4 (28/54/10/8%)
Number of lesions 1 / ≤ 3/ >3	33/11/6 (66/22/12%)
Size of largest HCC < 3 / ≥3 cm	3/47 (6/94%)
Vascular invasion: yes/ no	10/40 (20/80%)
Distant metastasis: yes/no	1/49 (2/98%)
CTP class: A/B/C	(4/16 /30)(8/32/60%)
BCLC stage: C/D	(20/30)(40/60%)
HCC grade: 1/2/3	(19/20 /11)(38/40/22%)
AFP: < 200/ ≥200 (ng /ml)	2/48(4/96%)

Table 3 shows that there were no significant differences between the 3 HCC grades regarding age; gender; residence; current smoking; presenting symptom; presence of jaundice; presence of DM or

hypertension. Patients with HCC grade 3 had significantly higher frequency of variceal bleeding than both grade 1 & 2. Patients with HCC grade 2 & 3 had significantly higher frequency of hepatic encephalopathy than grade 1. Patients with HCC grade 1 had significantly higher frequency of mild ascites than both grade 2 & 3. PST grade 1 was present in significantly higher frequency in HCC grade 1 than both grade 2 & 3.

Table 4 shows that circulating D-dimer, serum AFP & serum bilirubin levels were significantly higher in grade 2 and 3 HCC than grade 1 (p = 0.001, 0.001, 0.011 respectively), while serum albumin level was significantly lower in grade 2 and 3 HCC than grade 1. There was insignificant difference between the 3 grades regarding AST, ALT, hemoglobin level, white blood cells count, platelet count, international normalizing ratio (INR) and serum creatinine.

Table 5 shows significantly higher circulating D-dimer serum and AFP levels in BCLC stage D than BCLC stage C (p = 0.021, 0.001, respectively).

Table 6 shows a comparison of HCC cases within Milan criteria with HCC cases beyond Milan criteria. There were no significant differences between the two groups regarding age; gender; residence; current smoking; presenting symptom and history of diabetes or hypertension. There was a statistically significant higher frequency of hepatic encephalopathy in HCC grades beyond Milan criteria than those within Milan criteria. HCC grades beyond Milan had higher frequency of moderate & marked ascites and lesser frequency of mild ascites than HCC within Milan criteria. There was statistically significantly higher proportion of PST grade 1 in HCC grade within Milan than HCC grades beyond Milan criteria.

Table 7 shows that circulating D-dimer & serum AFP levels were significantly higher in HCC cases beyond Milan than HCC cases within Milan criteria (p=<0.0005, 0.001respectively). There were no significant differences between the 2 groups regarding serum bilirubin, serum albumin, AST, ALT, hemoglobin level, white blood cells count, platelet count, international normalizing ratio (INR) or serum creatinine.

Table 3 Clinical comparison of the three HCC grades

Variable	Grade 1 HCC (n=19)	Grade 2 HCC (n=20)	Grade 3 HCC (n=11)	P
Age (years)(Mean ± SD)	61.4±7	61.6±8	64.6±6.9	0.461
Gender (male/ female)	15/4 (78.9/21.1%)	17/3 (85/15%)	11/0 (100/0%)	0.274
Residence(urban/rural)	10/9 (52.6/47.4%)	8/12 (40/60%)	6/5 (54.5/45.5%)	0.649
Current smoking	8(42.1%)	7(35%)	5(45.5%)	0.827
Presenting symptom: Pain/ mass/both/no	16/1/2/0 (84.2/5.3/10.5/0%)	16/1/1/2 (80/5/5/10%)	8/0/1/2 (72.7/0/9.1/18.2%)	0.661
Bleeding varices	1 (5.3%)	1 (5%)	6 (54.5%)	<.0005
Hepatic encephalopathy	1 (5.3%)	4 (20%)	5 (45.5%)	0.03
Ascites: no/mild/moderate/ tense	2/11/6/0 (10.5/57.9/31.6/0%)	1/1/13/5 (5/5/65/25%)	1/3/5/2 (9.1/27.3/45.5/18.2%)	0.012
PST:grade1/2/3/4	11/8/0/0 (57.9/42.1/0/0%)	2/13/3/2 (10/65/15/10%)	1/6/2/2 (9.1/54.5/18.2/18.2%)	0.01
Diabetes	5 (26.3%)	6 (30%)	3(27.3%)	0.966
Hypertension	4 (21.1%)	4 (20%)	0(0%)	0.26

Table 4 Laboratory data of the studied patients in the different HCC grades

Variable	HCC grade			P
	Grade 1 (n=19)	Grade 2 (n=20)	Grade 3 (n=11)	
D-dimer(ng/ml)	200(0-400)	800(400-800)	400(400-800)	0.001
AFP(ng/ml)	311(234-355)	545(427- 656)	440(346-620)	0.001
Bilirubin(mg/dl)	2.2(1.8-2.7)	3(2.5-3.8)	2.7(2.5-3)	0.011
Albumin(gm/dl)	3(2.9-3.2)	2.7(2.5-2.9)	2.6(2.5-2.9)	0.002
ALT(IU/L)	44.4±25.4	57.6±34.2	53.3±20.6	0.354
AST(IU/L)	63(33-86)	58(46-69)	80(65-88)	0.143
Hemoglobin(gm/dl)	11.7±1.9	10.6±2.1	10.9±1.3	0.202
WBCs (109/L)	5.2(4.3-7.4)	6.75(5.3-10.9)	6.7(5.5-15.3)	0.059
Platelets (109/L)	125.7±53.4	147.8±63.8	134±65.7	0.522
INR	1.4(1.3-1.7)	1.62(1.4-1.7)	1.65(1.4-1.9)	0.28
Creatinine (mg/dl)	1.13±0.3	1.18±0.3	1.2±0.3	0.807

Table 5 Circulating D dimer and serum AFP levels in BCLC stage C HCC versus stage D

Variable	BCLC Stage C	BCLC Stage D	P
D-dimer (ng/ml)	400 (0-700)	800 (400-800)	0.021
AFP (ng/ml)	312.5 (231.8-437.5)	529 (368.5-658.8)	0.001

Table 6 Comparison of HCC cases within Milan criteria with HCC cases beyond Milan criteria

Variable	Milan criteria		P value
	Within (n=19)	Beyond (n=31)	
Age (years)(Mean ± SD)	61.4±7	62.7±7.7	0.559
Gender(male/ female)	15/4 (78.9/21.1%)	28/3 (90.3/9.7%)	0.237
Residence(urban/rural)	10/9 (52.6/47.4%)	14/17 (45.2/54.8%)	0.608
Current smoking	8 (42.1%)	12 (38.7%)	0.812
Presentation: Pain/ mass/both/no	16/1/2/0 (84.2/5.3/10.5/0%)	24/1/2/4 (77.4/3.2/6.5/12.9%)	0.721
History of bleeding varices	1(5.3%)	7(22.6%)	0.108
History of hepatic encephalopathy	1(5.3%)a	9(29%)b	0.042
Ascites: no/mild/moderate/ tense	2/11/6/0 (10.5/57.9/31.6/0%)	2/4/18/7 (6.5/12.9/58.1/22.6%)	0.003
PST:grade1/2/3/4	11/8/0/0 (57.9/42.1/0/0%)	3/19/5/4 (9.7/61.3/16.1/12.9%)	0.001
Diabetes	5(26.3%)	9(29%)	0.836
Hypertension	4 (21.1%)	4 (12.9%)	0.351

Table 7 Comparison of laboratory data of HCC cases within Milan criteria with HCC cases beyond Milan criteria

Parameter	HCC Milan criteria		P
	Within (n=19)	Beyond(n=31)	
D-dimer (ng/ml)	200(0:400)	800(400:800)	<.0005
AFP (ng/ml)	311(234:355)	544(413:650)	0.001
Bilirubin (mg/dl)	2.2(1.8:2.7)	2.9(2.5:3.5)	0.164
Albumin (mg/dl)	3(2.9:3.2)	2.7(2.5:2.9)	0.037
AST(IU/L)	63(33:86)	66(52:80)	0.849
ALT(IU/L)	44.4±25.4	56±29.8	0.164
Hemoglobin(mg/dl)	11.7±1.9	10.7±1.8	0.081
WBCs	5.2(4.3:7.4)	6.7(5.5:11.4)	0.498
Platelet count	130(85:153)	128(98:177)	0.832
INR	1.4(1.29:1.67)	1.63(1.38:1.7)	0.452
Creatinine (mg/dl)	1.13±0.3	1.18±0.3	0.533

Table 8 shows statistically significant positive correlation of circulating D-dimer level and both the largest tumor size and HCC grade (r = 0.352, 0.339, p = 0.012, 0.016 respectively).However, there was no significant correlation between circulating D-dimer level and number of HCC lesions, distant metastasis or vascular invasion separately.

Table 8 Correlation of circulating D-dimer level with HCC burden

Parameter	Correlation coefficient	P
Largest tumor size	0.352	0.012
Number of HCC lesions	0.136	0.346
HCC grade	0.339	0.016
Distant metastasis	-0.042	0.772
Vascular invasion	0.041	0.778

Figure 2 shows a statistically significant positive correlation of circulating D-dimer and AFP levels (r=0.451, p = 0.001)

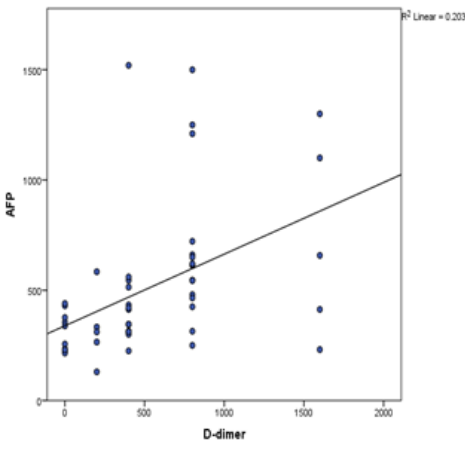

Figure 2 Correlation of circulating D-dimer and AFP levels.

Table 9 shows statistically significant positive correlation of both circulating D-dimer & serum AFP levels and ECOG PST (r = 0.4, p = 0.002).

Table 9 Correlation between both circulating D-dimer & serum AFP levels and ECOG performance status

Parameter	Correlation coefficient	P
AFP (ng/ml)	0.422	0.002
D-dimer (ng/ml)	0.423	0.002

Table 10 & Figure 3 shows that cutoff level of circulating D-dimer of ≥ 300 ng/ml can predict HCC grades beyond Milan with PPV of 77.1%; NPV of 73.3%; AUC of 0.800 and P value <0.0005.It also shows that serum level of AFP ≥394.5 ng/ml can predict HCC grades beyond Milan with PPV of 89.3%; NPV of 72.7%; AUC of 0.81 and P value <0.0005.

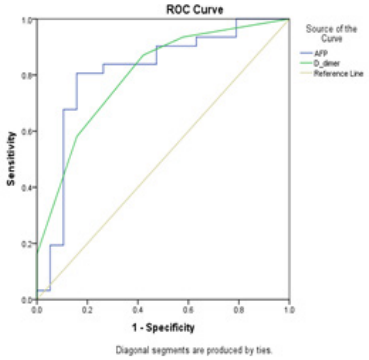

Figure 3 ROC curve for the prediction of HCC grades beyond Milan criteria using circulating D-dimer & serum AFP levels.

Table 10 Cutoff values of D-dimer and AFP for predicting HCC cases beyond Milan criteria (G2, 3)

Variable (ng/ml)	Cut-off	AUC (95% CI)	SE	P	Sn	Sp	PPV	NPV	AUC comparison	
									Z	P
D-dimer	≥ 300	.800(0.673:0..926)	0.065	<.0005	87.10%	57.90%	77.10%	73.30%	0.1	0.9166
AFP	≥394.5	.810(0.673:0.94)	0.07	<.0005	80.60%	84.20%	89.30%	72.70%		

Table 11 shows univariate logistic regression analysis to predict HCC grades beyond Milan criteria. Both circulating D-dimer & serum AFP levels at cutoff values of ≥300 ng/ml and ≥394.5 ng/ml, respectively, are predictors of HCC grades beyond Milan criteria (p=0.007, <0.0005 respectively).

Table 11 Univariate logistic regression analysis to predict HCC grades beyond Milan criteria

Predictor	B	P	OR (95% CI)
D-dimer ≥ 300 ng/ml	2.356	0.007	10.5 (1.9 - 57.6)
AFP ≥ 394.5 ng/ml	3.101	< .0005	22.2 (4.9 - 101.7)

Table 12 shows multivariate logistic regression analysis to predict HCC grades beyond Milan criteria. Serum AFP level at cutoff value of ≥394.5 ng/ml is an 'independent' predictor of HCC grades beyond Milan criteria (p = 0.001). This model is statistically significant and can classify 82% of cases correctly (p < .0005).

Table 12 Multivariate logistic regression analysis to predict HCC grades beyond Milan criteria

Variable	B	P	OR (95% CI)
D-dimer ≥ 300 ng/ml	1.384	0.101	4 (.764 - 20.8)
AFP ≥ 394.5 ng/ml	2.685	0.001	14.7 (3 - 71.7)

Discussion

Liver cancer is a global health problem with increasing incidence.[25,26] By 2025, more than one million patients will be affected by liver cancer annually.[27] HCC is the most common form of liver cancer and accounts for about 90% of cases. Its initiation and progression occur on top of chronic hepatitis, which creates a micro-environment favorable for tumor growth.[28] Leaky and dysfunctional blood vessels are formed in cirrhosis and HCC.[29,30,31] These blood vessels allow HCC cells to interact with the hemostatic system, thereby leading to the activation of the coagulation cascade.[32,33] So, both cirrhosis and liver cancer can change the hemostatic balance towards a hypercoagulable state that further influences tumor cell behavior.[34] Attention towards the relation between circulating D-dimer and tumor progression and invasion is increasing. The Aim of the present work was to study the correlation between circulating D-dimer level and the tumor grade in HCV related HCC. The present study showed that HCC was more prevalent in males, as shown by male to female ratio of 6:1. This is concomitant with that published in the literature.[35–38]

The possible explanation may be the differences in risk factors exposure, sex hormones and other genetic factors. It has been reported previously that androgens modulate hepatocarcinogenesis.[39] HCC cases in the present study were diagnosed at age of 62.2±7.4 years indicating that aging is a risk factor for HCC. The present study showed that HCC was uni-focal in 66 % of the cases; the size of largest tumor was ≥3 cm in 94% of cases; vascular invasion was present in 20%; 40% of the cases had BCLC stage C ;60% had BCLC stage D and 62% of HCC were beyond Milan criteria. This denotes that HCC is still diagnosed at late stages, indicating inadequate surveillance, which may be patient, health care provider or surveillance tool related factors. Recently, it is reported that 80% of patients with HCC are in advanced stage at the time of diagnosis.[40,41] HCC diagnosis at a late stage limits therapeutic choices, making it the third leading cause of cancer-related death worldwide.[42]

This study revealed that both synthetic and excretory functions of the liver, as evaluated by serum albumin and serum bilirubin, respectively, were more impaired in grade 2 and 3 HCC than grade 1 HCC. Previous study reported a statistically significant relationship between advanced HCC stage and both low serum albumin level and high serum bilirubin level.[43] In the present study, circulating D-dimer level was positivly correlated with HCC stage and size with higher level in advanced HCC stages. Previous studies have reported that elevated D-dimer level was associated with a heavier tumor burden.[24] Significant correlation of circulating D-dimer level with differentiation and TNM stage of HCC was also reported.[44] D-dimer was considerd as a predictors of poor prognosis for malignancy in cases of HCC, ovarian tumors, lung cancer and pancreatic cancer.[45] This may be explained by the release of procoagulant and fibrinolytic factors by HCC cells.[46] This leads to activation of both coagulation and fibrinolysis, which increase circulating D-dimer level.[45] The extent of this activation correlates with HCC stage and prognosis. The relationship between coagulation activation and tumor angiogenesis, progression, and metastatic spread has been previously reported.[9,10]

The present study showed that all HCC patients had performance status more than 0 and 72 % had performance status more than 1 at the time of diagnosis. PST grade 1 was present in significantly higher frequency in HCC grade 1 than both grade 2 and 3.It also showed that circulating D-dimer level was positivly correlated with ECOG PST of HCC patients. These findings can be explained by the fact that HCC patients with worse performance status had larger diameter of HCC and more frequent vascular invasion.[47] The present study showed that circulating D-dimer level at cut off ≥300 ng/ml can predict HCC cases beyond Milan criteria. Previous reports showed association between elevated preoperative D-dimer level and HCC recurrence.[24] So, circulating D-dimer level may help in the preoperative evaluation of HCC patients prepared for surgical treatments like liver resection and transplantation. The present work showed that there was also statistically significant positive correlation between circulating D-dimer and serum AFP level, a similar finding was previously reported.[24] This finding denotes that circulating D-dimer level is associated with the biological behavior of the tumor. Limitation of the present study is the late stages of HCC at the time of diagnosis where all the patients were diagnosed at either BCLC stage C or D, with absence of very early (BCLC stage 0), early (BCLC stage A) and intermediate (BCLC stage B) stage HCC. So, more studies including larger number of the different BCLC stages are recommended.

Conclusion

In HCV related HCC, circulating D-dimer level was positively correlated with HCC grade and largest tumor size with higher level in HCC grades 2&3 as well as BCLC stage D. Cut off value of circulating D-dimer level of ≥300 ng/ml can predict HCC grades beyond Milan criteria. Also, serum AFP level at cutoff value of ≥394.5 ng/ml was an 'independent' predictor of HCC grades beyond Milan criteria.

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None.

Conflicts of interest

The authors declare no conflicts of interest.

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