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ORIGINAL RESEARCH ARTICLE

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Study of the effect of vascular endothelial growth factor (*VEGF*) C(+405)G (rs2010963) single nucleotide polymorphism on the development of esophageal and gastric varices and risk of variceal bleeding in cirrhotic hepatitis C virus (HCV) patients (*VEGF*) C(+405)G IN esophageal and gastric varices

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Abstract

Background: HCV infection is a major worldwide cause of chronic liver diseases. Esophageal and gastric varices are common in cirrhotic patients due to concomitant portal hypertension. Variceal hemorrhage is a major decompensating event with high morbidity and mortality. Endothelial dysfunction, occurring in cirrhosis, facilitates the development of liver cirrhosis, portal hypertension and contributes to increased intrahepatic vascular resistance. VEGF family members are major regulators of blood vessel development and function.

Results: The study was conducted on 90 subjects admitted to Tropical Medicine Department, Alexandria Main University Hospital: 30 cirrhotic patients with endoscopically proven varices (group A), 30 cirrhotic patients without varices (group B), and 30 healthy controls (group C). All patients was subjected to detailed history taking and thorough clinical examination, laboratory investigations, ultrasound abdomen, upper gastrointestinal endoscopy, and genotyping for *VEGF* C(+405)G (rs2010963) by 5′ nuclease assay. The *VEGF* C(+405)G (rs2010963) GG genotype was associated with higher prevalence of esophageal and gastric varices and higher bleeding risk.

Conclusion: *VEGF* C(+405)G (rs2010963) is an important genetic determinant of esophageal varices, gastric varices, and correlates with variceal bleeding risk. Genetic testing of this SNP would be useful in prediction of esophageal and gastric varices and bleeding risk.

Keywords: *VEGF*, Varices, Variceal bleeding, Cirrhosis

Background

HCV infection is a worldwide cause of chronic liver diseases [1]. The long-term progression of HCV infection is highly variable. The hepatic pathology progress from minimal histological alterations up to extensive hepatocellular necrosis, fibrosis, and cirrhosis that could be complicated by hepatocellular carcinoma (HCC) [2]. In

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Egypt, the results of national survey conducted by the Egyptian ministry of health showed that HCV prevalence in those over 18 years was about 4% [3].

Cirrhosis is the common end-result for chronic liver injury regardless of the etiology. Histologically, it is characterized by diffuse hepatocellular necrosis and diffuse nodular regeneration surrounded by dense fibrotic septa causing pronounced distortion of hepatic vascular architecture causing increased resistance to portal blood flow and hence in portal hypertension [4].

Esophageal and gastric varices are present in about 50% of patients with cirrhosis, and hepatic decompensation is linked to higher risk of variceal formation and bleeding [5, 6]. Variceal hemorrhage occurs at a rate of around 10-15% per year and depends upon the severity of liver disease, degree of portal hypertension, size and location of varices, and bleeding risk signs, e.g., red wale marks, cherry spot, and varices on varices. Six-week mortality is still high (15-25%) even with advanced medical and endoscopic treatment [7, 8].

Portal hypertension in cirrhosis occurs due to both the increase in intrahepatic vascular resistance (static element) and the increase in blood flow in the splanchnic circulation (kinetic element) [9].

Endothelial dysfunction, occurring in cirrhosis, leads to impaired vasomotor control, inflammation, fibrosis, and impaired liver regeneration [10, 11]. All of which enhance progression of liver cirrhosis and portal hypertension. It also leads to increased vasodilators especially nitric oxide (NO) which is the most potent vasodilator molecule known [12, 13].

Members of the VEGF family are major regulators of blood vessels growth and function [14]. VEGF also regulates endothelial cell survival and apoptosis [15–17]. VEGF is the major factor to endothelial proliferation and neoangiogenesis [18]. Activation of VEGF receptor leads to eNOS activation and release [19]. VEGF is also a potent inducer of vascular permeability and inflammation [20].

Aim of the work

The aim of the work was the assessment of effect of *VEGF* C(+405)G (rs2010963) single nucleotide polymorphism (SNP) on development and grade of esophageal and gastric varices and risk of bleeding in cirrhotic patients with HCV.

Subjects

The study was conducted on 90 subjects, 30 cirrhotic patients with varices on endoscopy (group A), 30 cirrhotic patients without varices on endoscopy (group B), and 30 healthy controls (group C). All subjects with renal impairment, hepatocellular carcinoma (HCC), or other malignancy, on beta-blockers, non-steroidal anti-inflammatory drugs or anticoagulant, patients with platelet count < 50000 cell/cmm or international normalized ratio > 1.5, and patients with any other source of gastric bleeding (ulcer, portal hypertensive gastropathy) were excluded.

Methods

An informed consent was obtained from all participants before enrollment. Based on 800 cirrhotic patients with HCV being admitted to Tropical Medicine Department, Alexandria Main University Hospital annually and there was only 2 previous studies performed on *VEGF* C(+405)G (rs2010963) in Egyptian patients with the prevalence of GG genotype was about 6.7% and 5.5% with precision of 5 and α of 5% [21–23]. The minimum number needed for our study was calculated to be 86 patients [24]. All subjects were submitted to the following, detailed history taking and thorough clinical examination including abdominal examination, laboratory investigations including complete blood picture (CBC), erythrocyte sedimentation rate (ESR), C reactive protein (CRP), liver functions tests (serum bilirubin (total, direct), albumin, international normalized ratio (INR), liver enzymes (aspartate transferase (AST), alanine transferase (ALT), alkaline phosphatase (ALP)) renal functions tests (blood urea and creatinine), alfa-fetoprotein, HCV antibodies, or polymerase chain reaction (PCR), hepatitis B surface antigen (HBs Ag), AST to platelet ratio index (APRI) score, ultrasound, gastroduodenoscopy for variceal detection, and grading and genotyping for (*VEGF*) C(+405)G (rs2010963) using allelic discrimination 5′ nuclease assay. Data were fed to the computer and analyzed using the IBM SPSS software package version 20.0. Comparisons between groups for categorical variables were assessed using Chi-square test (Fisher or Monte Carlo). *F* test (ANOVA) for normally distributed quantitative variables, to compare between more than two groups, and post hoc test (LSD) for pairwise comparisons. Kruskal-Wallis test for abnormally distributed quantitative variables, to compare between more than two studied groups, and post hoc (Dunn's multiple comparisons test) for pairwise comparisons. Significance of the obtained results was judged at the 5% level.

Results

The current study involved 90 participants divided into 3 groups: group A, 30 patients with HCV-related liver cirrhosis and esophageal varices; group B, 30 patients with HCV-related liver cirrhosis with no esophageal varices group C, 30 apparently healthy controls.

There was no statistically significant difference between the studied groups as regard age ($p = 0.749$)

Table 1 Comparison between the studied groups according to demographic data, complete blood count, creatinine, ALT, AST, ALP, bilirubin, INR, albumin, splenic bipolar diameter, and portal vein diameter by ultrasound abdomen and APRI

	Group A ($n = 30$)	Group B ($n = 30$)	Group C ($n = 30$)	p
Sex				
Males	19	63.3	18	0.725
Females	11	36.7	12	
Age (years)				
Min.-max.	37.0-78.0	37.0-75.0	42.0-77.0	0.749
Mean ± SD	64.70 ± 8.65	62.90 ± 8.54	64.07 ± 10.51	
Hemoglobin (g/dL)				
Min.-max.	4.50-12.80	9.50-14.50	10.10-17.70	< 0.001*
Mean ± SD	8.81 ± 2.26	12.10 ± 1.34	13.92 ± 1.77	
Sig. bet. grps.	$p_1 < 0.001$*, $p_2 < 0.001$*, $p_3 = 0.001$*			
WBCs (cell/cmm)				
Min.-max.	1.80-13.0	2.10-7.45	4.30-10.40	< 0.001*
Mean ± SD	3.58 ± 2.38	4.23 ± 1.23	7.12 ± 1.37	
Sig. bet. grps.	$p_1 = 0.323$, $p_2 < 0.001$*, $p_3 < 0.001$*			
Platelets (cell/cmm)				
Min.-max.	53.0-140.0	55.0-145.0	164.0-415.0	< 0.001*
Mean ± SD	78.80 ± 26.16	117.73 ± 20.08	275.23 ± 66.26	
Sig. bet. grps.	$p_1 = 0.002$*, $p_2 < 0.001$*, $p_3 < 0.001$*			
Creatinine (mg/dl)				
Min.-max.	0.50-1.40	0.50-1.30	0.40-1.20	0.189
Median (IQR)	1.10 (0.70-1.20)	0.85 (0.70-1.10)	0.85 (0.80-1.10)	
ALT (U/L)				
Min.-max.	18.0-153.0	19.0-113.0	11.0-65.0	< 0.001*
Median (IQR)	55.50 (45.0-67.0)	35.0 (27.0-65.0)	18.50 (16.0-21.0)	
Sig. bet. grps.	$p_1 = 0.301$, $p_2 < 0.001$*, $p_3 < 0.001$*			
AST (U/L)				
Min.-max.	23.0-112.0	18.0-118.0	13.0-43.0	< 0.001*
Median (IQR)	59.50(38.0-87.0)	44.50(39.0-78.0)	22.0(17.0-27.0)	
Sig. bet. grps.	$p_1 = 0.411$, $p_2 < 0.001$*, $p_3 < 0.001$*			
ALP (U/L)				
Min.-max.	103.0-198.0	78.0-236.0	44.0-137.0	< 0.001*
Mean ± SD	158.37 ± 27.76	126.97 ± 39.92	79.17 ± 24.03	
Sig. bet. grps.	$p_1 = 0.001$*, $p_2 < 0.001$*, $p_3 < 0.001$*			
Bilirubin (mg/dL)				
Min.-max.	0.80-22.0	0.80-6.70	0.40-1.20	< 0.001*
Median (IQR)	2.30 (1.90-3.20)	1.60 (1.30-2.10)	0.75 (0.60-0.90)	
Sig. bet. grps.	$p_1 = 0.021$*, $p_2 < 0.001$*, $p_3 < 0.001$*			
INR				
Min.-max.	1.0-1.40	1.0-1.40	0.80-1.30	< 0.001*
Mean ± SD	1.28 ± 0.12	1.14 ± 0.13	1.0 ± 0.10	
Sig. bet. grps.	$p_1 < 0.001$*, $p_2 < 0.001$*, $p_3 < 0.001$*			
Albumin (g/dL)				
Min.-max.	1.70-3.60	2.20-4.24	3.50-5.20	< 0.001*
Median (IQR)	2.70 (2.30-2.90)	3.05 (2.70-3.30)	3.90 (3.70-4.50)	
Sig. bet. grps.	$p_1 = 0.001$*, $p_2 < 0.001$*, $p_3 < 0.001$*			
Splenic span				
Min.-max.	16.0-23.0	15.0-19.0	9.0-12.50	< 0.001*
Mean ± SD	19.0 ± 1.97	16.50 ± 1.18	10.82 ± 0.75	

Table 1 (continued)

	Group A ($n = 30$)	Group B ($n = 30$)	Group C ($n = 30$)	p
Sig. bet. grps.	$p_1 < 0.001$*, $p_2 < 0.001$*, $p_3 < 0.001$*			
Portal vein diameter				
Min.-max.	11.0-19.0	10.0-16.0	7.0-12.0	< 0.001*
Mean ± SD	14.63 ± 1.67	13.42 ± 1.32	9.30 ± 1.26	
Sig. bet. grps.	$p_1 = 0.004$*, $p_2 < 0.001$*, $p_3 < 0.001$*			
APRI				
Min.-max.	0.69-5.28	0.33-2.32	0.09-0.35	< 0.001*
Mean ± SD	2.21 ± 1.04	1.21 ± 0.56	0.22 ± 0.08	
Sig. bet. grps.	$p_1 < 0.001$*, $p_2 < 0.001$*, $p_3 < 0.001$*			

Group A, 30 cases of cirrhosis with esophageal varices

Group B, 30 cases of cirrhosis without esophageal varices

Group C 30 healthy individuals as controls

χ^2 Chi square test, *F F* for ANOVA test, *IQR* interquartile range, *p p* value for comparing between the studied groups

Table 2 Comparison between groups A and B according to Child-Pugh classification and score

	Group A ($n = 30$)	Group B ($n = 30$)	p
Child-Pugh class			
A	4	13	< 0.001*
B	9	11	
C	17	6	
Child-Pugh score			
Min.-max.	5.0-15.0	5.0-11.0	< 0.001*
Mean ± SD	9.57 ± 2.60	7.17 ± 1.66	

Group A, 30 cases of cirrhosis with esophageal varices

Group B, 30 cases of cirrhosis without esophageal varices

t Student *t* test, *IQR* interquartile range, *p p* value for comparing between the studied groups

*Statistically significant at $p \leq 0.05$

and sex ($p = 0.725$) (Table 1). The results of complete blood count, creatinine, ALT, AST, ALP, bilirubin, INR, albumin, splenic bipolar diameter, and portal vein diameter by ultrasound abdomen and APRI; all are summarized in Table 1.

The mean of Child-Pugh score was statistically significantly higher in cirrhotic patients with varices (mean 9.57) than cirrhotic patients with no varices (mean 7.17) ($P < 0.001$) (Table 2).

Endoscopically, using Paquet's classification for grading of esophageal varices, the most frequent grade was IV (15 patients (50%)), followed by grade 1 with 6 patients (20%), grade 2 in 5 patients (16.7%), and grade 3 in 4 patients (13.3%) [7]. Gastric varices were present in 11 patients (36.7%). Twenty-two patients (73.3%) had endoscopic risk signs of variceal bleeding (white nipple, red wales, clots overlying a varix, cherry red spot, or varix on varix) [25]. Endoscopic therapy (band ligation) had been accomplished in 20 patients (66.7%).

The genotype distribution of *VEGF* C(+405)G (rs2010963) SNP in all groups was in accordance with Hardy-Weinberg equilibrium [26] (Table 3).

The GG genotype was statistically significantly more frequent in cirrhotic patients with esophageal varices than cirrhotic patients without esophageal varices and controls ($P < 0.001$). There was no statistically significant difference between the studied groups as regard the CG genotype ($P = 0.866$). The G allele was statistically significantly more frequent in group A than group B and group C ($P < 0.001$). There was no statistically significant difference between group B and controls as regard both C and G alleles ($P = 0.838$) (Table 3) (Fig. 1).

Cirrhotic patients with GG genotype displayed 14.857-fold increased risk for developing esophageal varices than CC genotype and patients with CG genotype displayed 1.905-fold increased risk for developing esophageal varices than patients with CC genotype, while cirrhotic patients carrying G allele had 4.125-fold increased risk for esophageal varices than patient carrying C allele (Table 4).

Cirrhotic patients with varices (group A) who had the GG genotype had statistically significantly higher grade of esophageal varices, more frequent gastric varices, more evident risk signs of bleeding, and more frequent variceal bleeding when compared to those who had the CC and CG genotype (Table 5).

Cirrhotic patients (group A, B) who had the GG genotype had statistically significantly higher APRI, higher Child-Pugh score, and larger portal vein diameter when compared to those who had the CC and CG genotype (Table 6).

Table 3 Comparison between the three studied groups according to (*VEGF*) C(+405)G (rs2010963) single nucleotide polymorphism and whether observed genotype frequencies are consistent with Hardy-Weinberg equilibrium [23]

	Group ($n = 30$)		Group B ($n = 30$)		Group C ($n = 30$)		χ^2	p
	No.	%	No.	%	No.	%		
VEGF gene								
CC	7	23.3	16	53.3	16	53.3	7.330*	0.026*
CG	10	33.3	12	40.0	11	36.7	0.287	0.866
GG	13	43.3	2	6.7	3	10.0	15.417*	< 0.001*
HWE	**0.094**		**0.901**		**0.595**			
Allele								
C	24	40.0	44	73.3	43	71.7	17.908*	< 0.001*
G	36	60.0	16	26.7	17	28.3		
Sig. bet. grps.	$p1 < 0.001$*, $p2 < 0.001$*, $p3 = 0.838$							

Group A, 30 cases of cirrhosis with esophageal varices

Group B, 30 cases of cirrhosis without esophageal varices

Group C, 30 healthy individuals as controls

If $p < 0.05$—not consistent with HWE

Not accurate if < 5 individuals in any genotype group

χ^2 Chi square test, *MC* Monte Carlo, *p p* value for comparing between the studied groups

*Statistically significant at $p \leq 0.05$

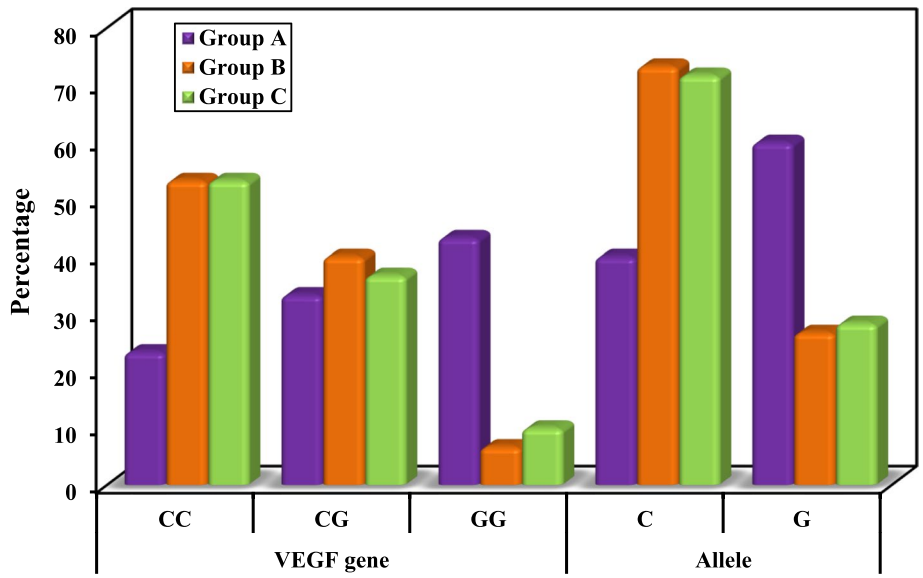

Fig. 1 Comparison between the three studied groups according to VEGF C(+405)G (RS2010963) SNP in the studied groups ($n = 90$)

Discussion

VEGF is produced by the endothelial cells, the macrophages, the T cells, the platelets, and many other cell types. Many researchers outlined the key role of VEGF in many vascular diseases as important pro-angiogenic factor regulating the normal and pathological angiogenic processes [27–31]. Many studies had demonstrated that VEGF had a pivotal role in pathogenesis and progression of portal hypertension in cirrhosis through potentiation of inflammation and enhancement of portal-systemic collateral vessel formation [32–38]. Watson et al. studied promotor and 5 untranslated region of VEGF gene and found that the GG genotype of *VEGF* C(+405) G (rs2010963) SNP was associated with higher serum VEGF production level than the CG and CC genotypes [39].

Table 4 VEGF C(+405)G (rs2010963) SNP genotype and risk for esophageal varices

	Group ($n = 30$)		Group B ($n = 30$)		*p*	OR (95% C.I.)
	No.	%	No.	%		
VEGF genotype						
CC	7	23.3	16	53.3		1.000
CG	10	33.3	12	40.0	0.301	1.905 (0.561-6.464)
GG	13	43.3	2	6.7	0.002*	14.857 (2.625-84.100)
Allele						
C	24	40.0	44	73.3		1.000
G	36	60.0	16	26.7	< 0.001*	4.125 (1.908-8.916)

Group A, cirrhosis with esophageal varices

Group B, cirrhosis without esophageal varices

OR odd's ratio, *C.I.* confidence interval, *LL* lower limit, *UL* upper limit

*Statistically significant at $p \leq 0.05$n

Table 5 Relation between (*VEGF*) C(+405)G (rs2010963) SNP with grade of esophageal varices, risk signs of variceal bleeding, gastric varices, and variceal bleeding in group A

	VEGF gene						^{MC}p
	CC ($n = 7$)		CG ($n = 10$)		GG ($n = 13$)		
	No.	%	No.	%	No.	%	
Grade of esophageal varices							
I	4	57.1	2	20.0	0	0.0	< 0.001*
II	2	28.6	3	30.0	0	0.0	
III	1	14.3	2	20.0	1	7.7	
IV	0	0.0	3	30.0	12	92.3	
Risk signs of variceal bleeding							
No	4	57.1	4	40.0	0	0.0	MCp = 0.004*
Yes	3	42.9	6	60.0	13	100.0	
Gastric varices							
No	7	100.0	8	80.0	4	30.8	0.003*
Yes	0	0.0	2	20.0	9	69.2	
Variceal bleeding							
No	5	71.4	7	70.0	2	15.4	0.009*
Yes	2	28.6	3	30.0	11	84.6	

χ^2 Chi square test, *MC* Monte Carlo, *p p* value for comparing between different categories

*Statistically significant at $p \leq 0.05$

Our results are in ordinance with the previous study performed by Yang et al. who demonstrated the GG genotype of *VEGF* C(+405)G (rs2010963) SNP was associated with higher incidence of esophageal varices though the patients included were Taiwanese, and different etiologic factors of cirrhosis were included with HBV being the most prominent factor; the odd ratio for the GG genotype was lower than the current study (3.1 for the GG genotype) and no increased risk of variceal bleeding was demonstrated [40].

The more frequent and larger esophageal varices, the more frequent gastric varices and the more frequent variceal bleeding noticed with the GG genotype in the current study could be attained to the fact that this genotype is associated with higher VEGF production [39]. The higher VEGF production enhances angiogenesis [27] leading to more portal collateral circulation [38, 41, 42] and that VEGF is also associated with unregulated hepatic inflammation leading to more cirrhosis progression and higher portal pressure [36–38].

Lower serum level of VEGF in cirrhotic patients was shown by Assay et al. who attained it to endothelial dysfunction caused by cirrhosis [36]. Huang et al. hypothesized that the higher levels of serum VEGF in

Table 6 Relation between (*VEGF*) C(+405)G (rs2010963) single nucleotide polymorphism gene with APRI, Child score, and portal vein diameter in group A + B ($n = 60$)

	VEGF gene			Test of Sig.	*p*
	CC ($n = 23$)	CG ($n = 22$)	GG ($n = 15$)		
Child score					
Min.-max.	5.0-12.0	5.0-12.0	5.0-15.0	$F = 5.474$*	0.007*
Mean ± SD	7.70 ± 1.82	7.91 ± 2.0	10.07 ± 3.24		
Median	8.0	8.0	11.0		
Sig. bet. grps.	$p_1 = 0.948, p_2 = 0.008$*, $p_3 = 0.019$*				
APRI					
Min.-max.	0.33-3.60	0.69-4.23	0.89-5.28	$F = 4.006$*	0.024*
Mean ± SD	1.45 ± 0.78	1.59 ± 0.86	2.29 ± 1.19		
Median	1.16	1.44	2.37		
Sig. bet. grps.	$p_1 = 0.862, p_2 = 0.023$*, $p_3 = 0.073$				
Child score					
Min.-max.	5.0-12.0	5.0-12.0	5.0-15.0	$F = 5.474$*	0.007*
Mean ± SD	7.70 ± 1.82	7.91 ± 2.0	10.07 ± 3.24		
Median	8.0	8.0	11.0		
Sig. bet. grps.	$p_1 = 0.948, p_2 = 0.008$*, $p_3 = 0.019$*				
Portal vein diameter (mm)					
Min.-max.	10.0-16.0	11.0-16.0	12.0-19.0	4.749*	0.012*
Mean ± SD	13.60 ± 1.49	13.77 ± 1.45	15.07 ± 1.67		
Median	14.0	14.0	15.0		
Sig. bet. grps.	$p_1 = 0.919, p_2 = 0.014$*, $p_3 = 0.036$*				

F F for ANOVA test, *H H* for Kruskal-Wallis test, *p p* value for comparing between different categories

early stages of cirrhosis were due to homeostatic compensatory mechanisms [41]. Desideri et al., Akiyoshi et al., and Robinson et al. noticed that serum VEGF levels decreases as cirrhosis progresses in accordance with the Child–Pugh classification and is influenced by complex factors including etiology of liver disease, platelets dysfunction, and different VEGF isoforms [43–45]. All those studied could explain the lower urinary levels of VEGF in cirrhotic patients with esophageal varices than those without demonstrated by Mohamed et al. Low VEGF level could be attained to higher degree endothelial dysfunction and lower platelets count in cirrhotic patients with varices than those without [46]. Those studies also points out to the value of *VEGF* gene polymorphism over serum level to predict variceal occurrence in cirrhotic patients.

Applying those finding, we could conclude that the GG allele is associated with a better VEGF response to incentive stimuli causing higher VEGF production and effect of VEGF causing enhanced growth of compensatory collateral circulation which facilitates esophageal and gastric varices development and progression and hence higher risk of variceal bleeding. *VEGF* C(+405) G (rs2010963) SNP is a better marker to predict esophageal varices occurrence over the VEGF serum level which is affected by many factors including the stage and etiology of cirrhosis. The current study highlights the importance of individualized medical care based on genomic sequencing which would hopefully be more widespread and less expensive in the near future.

Limitations

This is a single center experience; the patients were only Caucasians and single SNP was targeted.

Conclusion

VEGF C(+405)G (rs2010963) SNP plays a major role in development of esophageal varices, gastric varices, and risk of variceal bleeding. It also plays a key role in portal hypertension pathogensis and progression.

Abbreviations

VEGF: Vascular endothelial growth factor; HCV: Hepatitis C virus; SNP: Single nucleotide polymorphism; NO: Nitric oxide; HCC: Hepatocellular carcinoma; AST: Aspartate transferase; ALT: Alanine transferase; ALP: Alkaline phosphatase; CBC: Complete blood count; ESR: Erythrocyte sedimentation rate; CRP: C reactive protein; PCR: Polymerase chain reaction; HBV: Hepatitis B virus; HBs Ag: Hepatitis B surface antigen; APRI: AST to platelet ratio index; AFP: Alfa-fetoprotein.

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Authors' contributions
N A, M E initiated the project, designed the study, and was responsible for the concept. E T, R A shared in the literature review and writing the manuscript. A A, R A shared in data collection. All authors provided critical feedback and helped shape and revise the research, the analysis, and the manuscript. All authors have read and approved the manuscript.

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Availability of data and materials
Data are available with corresponding author to be presented upon request.

Declarations

Ethics approval and consent to participate
This study had been performed in accordance with the ethical standards. Faculty of Medicine, Alexandria University Ethical Committee approval held at 19 August 2019 before starting the study, and the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki. A written consent was obtained from each participant. Committee's serial number is 0201264 and reference number is FWA NO: 00018699.

Consent for publication
Written informed consents were obtained from both patients and control. Patient involved in this study agree for publication of data.

Competing interests
The authors declare that they have no competing interests.

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