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Influence of Heme Oxygenase- 1 Polymorphism (Rs2071746) on Esophageal Varices among Patients with Cirrhosis

Ahmed Ahmed El-Shaarawy[1], Eman Ahmed Gawish[1], Ahmed Kamal Khamis[2], Omnia Salah Nabih Ahmed*[1], Mary Albert Naguib[1]

Departments of [1]Clinical Pathology, [2]Hepatology, National Liver Institute, Menoufia University, Menoufia, Egypt

***Corresponding author:** Omnia Salah Nabih Ahmed, **Mobile:** (+20)01066113510, **E-mail:** dromniasalah@yahoo.com

ABSTRACT

Background: Portal hypertension (PHT) is a frequent clinical syndrome arising as a sequela of mesenchymal dysfunction in a cirrhotic liver. Esophageal varices (EV) are portosystemic collaterals in the submucosa of the lower esophagus. Heme oxygenase -1 (HO-1) was suggested as having vascular effects. So, in our study, we aimed to evaluate the role of HO-1 promotor polymorphism (rs2071746) on esophageal varices among patients with cirrhosis.

Objectives: The present study was designed to evaluate the role of HO-1 SNP 413A>T promoter (rs2071746) polymorphism in the development of esophageal varices among cirrhotic patients.

Patients and methods: A total of 105 subjects, including 35 cirrhotic patients with esophageal varices, 35 cirrhotic patients without esophageal varices, and 35 healthy controls were selected. HO-1 rs2071746 polymorphism was studied using Real-Time polymerase chain reaction (Real-Time PCR).

Results: we found that AA, AT, AA+AT genotypes were significantly associated with an elevated risk of EV development (OR =6.82, 95% CI =1.24- 37.54), (OR= 6.43, 95% CI =1.21- 34.19), (OR= 6.60, 95% CI=1.33- 32.84) respectively. And, the A allele was correlated with an increased risk of EV (OR= 2.06, 95% CI=1.40- 4.10). Patients with the AA genotype were significantly associated with lower platelet levels (P= 0.026), lower platelets count/ spleen diameter ratio (p-value= 0.014). Also, they had large varices (p-value= 0.025).

Conclusion: Our results suggest rs2071746 polymorphism could be used as a potential predictor and early diagnostic marker of EV development.

Keywords: Esophageal Varices, Heme Oxygenase -1, Polymorphism.

INTRODUCTION

Cirrhosis is a diffuse morphology that is characterized by excess fibrous tissue circumscribing parenchymal nodules, which consist of regenerating hepatocytes and alterations in hepatic vascular architecture. Liver cirrhosis (LC) is the twelfth leading cause of death worldwide [(1)].

Hepatitis C more than hepatitis B has emerged as the leading cause of chronic hepatitis and cirrhosis. Many cases of the formally called cryptogenic cirrhosis appear to be related to nonalcoholic fatty liver disease [(2)].

Portal hypertension (PHT) is one of the most important sequelae of cirrhosis leading to clinically relevant outcomes in terms of varices, ascites, splenomegaly hepatopulmonary syndrome, and hepatorenal syndrome. PHT is defined as a hepatic venous pressure gradient greater than 5 mmHg, with clinical consequences arising once this exceeds 10 mmHg [(3)].

Esophageal varices (EV) are portosystemic collaterals that form because of PHT preferentially in the submucosa of the lower esophagus. Variceal bleeding accounts for 10–30% of all cases of upper gastrointestinal bleeding. EV can be graded based on diameter size and are classified as small or large at a cut-off of 5 mm (North Italian Endoscopy Club classification) and accordingly, they can be managed either medically and/or endoscopically [(4)].

Multiple factors were encountered as predictors of EV development and bleeding. Low platelet count, spleen size >13.8 mm, portal vein >13 mm, splenic vein >11.5 mm, and a platelet spleen diameter ratio cutoff of 909 were found to be predictors of large EVs [(5)].

The major cause of portal hypertension in cirrhosis is increasing intrahepatic vascular resistance associated with fibrosis/cirrhosis. Nitric oxide (NO), the most powerful vasodilator substance, is significantly reduced in cirrhotic livers [(6)].

Heme oxygenase -1 (HO1) is a stress-responsive enzyme that catabolizes the heme resulting in the formation of equivalent amounts of ferrous iron (Fe2+), biliverdin (BV), and carbon monoxide (CO). These substances have a cytoprotective mechanism against oxidative stress occurring in the vascular system [(7)].

The up-regulation of HO-1 could inhibit the activity of the NO synthase enzyme through multiple mechanisms, as CO product could serve as a potent inhibitor of the enzyme reaction resulting in suppressing the potent NO-mediated biological actions such as vasorelaxation and lowering intrasinusoidal resistance occurring in cirrhosis [(8)].

In addition, the HO-1 reaction requires electrons from reduced nicotinamide adenine dinucleotide phosphate (NADPH) which competes for them with NO synthase [(9)].

Variation in genes might play a role in the development of esophageal varices and their risk of bleeding. This fact could improve the selection of candidates for esophagogastroduodenoscopy and could help in a detailed understanding of the pathogenesis of esophageal varices and thereafter, the prognosis of liver cirrhosis [(10)].

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Polymorphisms in the promoter region of the HO-1 gene might modulate the transcriptional level of HO-1 and have significant associations with human diseases related to the vascular system [(11)].

The present study was designed to evaluate the role of HO-1 SNP 413A>T promoter (rs2071746) polymorphism in the development of esophageal varices among cirrhotic patients.

PATIENTS AND METHODS

The present study was carried out at Laboratory Medicine Department, National Liver Institute, Menoufia University, Egypt from March 2020 to February 2021.

A total of 105 subjects were enrolled in this case-control study, including 35 cirrhotic patients (LC) with varices, 35 cirrhotic patients without varices, and 35 healthy individuals matched in age and sex as a control group. Fibroscan was done to confirm cirrhotic liver. Duplex ultrasound of the liver and upper gastrointestinal endoscopy were used to assess different grades of esophageal varices using North Italian Endoscopy Club classification for grading of esophageal varices. Also, platelet count/spleen diameter ratio was calculated as a screening tool for prediction for EV presence using equation (platelet count (N/mm3)/Spleen diameter (mm)), patients with ratio ≤ 909 were most likely to have EV while patients with a ratio ≥ 909 counts were most likely to be free from EV [(12)].

Ethical consent:

The study protocol was approved by the local ethics committee of The National Liver Institute, Menoufia University. Informed consent was taken from both the patients and control group subjects after explaining the aim and concerns of the study. This work has been carried out following The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

For all subjects, the following were done: a collection of relevant clinical data, basic laboratory tests including Liver function tests (Cobas-6000 auto analyser, Roche Diagnostics, Germany), prothrombin time (Coagulometer CA -1500, Siemens, Germany). Hepatitis serology (HBs Ag and HCV Ab) (Cobas e411 immunoassay analyzer, Roche Diagnostics, Germany). Molecular testing for HO-1 polymorphism (rs2071746) by real-time PCR assay.

DNA extraction and genotyping:

Total DNA was extracted from an EDTA-treated blood sample using the Thermo Scientific Gene JET Whole Blood Genomic DNA Purification Mini Kit (QIAGEN, Chicago, USA).

The HO-1 T(-413)A promoter SNP (rs2071746) genotyping was performed by Rotor-Gene Q real-time PCR system (QIAGEN) using TaqMan ABI allelic discrimination kit (catalog #4351379, assay ID C-15869717-10, Applied Biosystems, Chicago, USA). The 40 × SNP genotyping assay was diluted to a 20 × working stock solution, of which. 5 µl was added to 10 µl TaqMan Universal PCR Master Mix (Chicago, USA), 7.5 µl of the extracted genomic DNA and 2 ul nuclease-free H_2O in a reaction total volume of 20 µl. One probe is labeled with VIC as the reporter dye to detect the A allele, while the other probe is labeled with FAM as the reporter dye to detect the T allele.

PCR cycling conditions: An initial denaturation step at 95°C for 10 min, followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 second. Then final extension at 60°C for 5 min was carried out. As a negative control, a PCR mix without a DNA sample was used to ensure a non-contaminated PCR product. Allelic discrimination plate read was analyzed and the allele types were verified.

Statistical analysis

Statistical analysis of the present study was conducted using SPSS version 22.0 (SPSS Inc., Chicago, IL, USA). Data was expressed into two phases: Descriptive and analytical study Chi-square test, Kruskal–Wallis test, Fisher's Exact test, odds ratio (OR), and confidence interval (CI) test were used. P-value > 0.05 was considered statistically non-significant. P-value < 0.05 was considered statistically significant. P-value 0.000 (<0.001) was considered statistically highly significant.

RESULTS

Baseline characteristics of the study subjects:

There was no significant difference between the three statistically studied groups as regard age (P=0.08) & gender (P=0.395). Compared to cirrhotic patients without EV, cirrhotic patients with EV had significantly higher levels of aspartate aminotransferase (AST), alkaline phosphatase, total and direct bilirubin, and significantly lower levels of hemoglobin (Hb) and platelets **(Table 1).**

Table (1): Demographic data and laboratory parameters of patients had (LC) with and without (EV) and healthy controls:

Laboratory parameters	G I LC with EV (n= 35)	G II LC without EV (n= 35)	G III Healthy controls (n= 35)	Significance test	Pairwise comparisons
Age (years) Median (IQR) Range (min-max)	57.00 (14.00) 38.00 - 71.00	55.00 (12.00) 40.00 - 70.00	53.00 (8.00) 42.00 - 66.00	□□= 5.06[a] P-value =0.080 NS	-
Gender (n (%)) Male Female	23 (65.7) 12 (34.3)	22 (62.9) 13 (37.1)	27 (77.1) 8 (22.9)	□□= 1.86 [b] P-value =0.395 NS	-
AST (U/L) Mean ± SD	47.00 ±1.45	37.00 ± 5.36	15.00 ±2.98	χ2= 56.07 [a] P-value <0.001 HS	p1=0.041 S p2<0.001 HS p3<0.001 HS
ALT (U/L) Mean ± SD	20.00 ±2.59	17.00 ±2.22	16.00 ±2.26	χ^2= 4.77 [a] P-value =0.092	-
ALP (U/L) Mean ± SD	90.00 ± 20.81	74.00 ±16.51	66.00 ± 13.21	χ^2= 18.08 [a] **P-value <0.001 HS**	**p1=0.027 S** **p2<0.001 HS** p3=0.172 NS
GGT (U/L) Mean ± SD	52.00 ± 12.13	30.00 ± 4.81	19.00 ±4.81	χ^2= 28.34 [a] **P-value <0.001 HS**	p1=0.051 NS **p2<0.001 HS** **p3<0.001 HS**
Total bilirubin (mg/dL) Mean ± SD	2.00 ±0.42	1.00±0.31	0.54±0.11	χ^2= 54.86 [a] **P-value <0.001 HS**	**p1<0.001 HS** **p2<0.001 HS** **p3<0.001 HS**
Direct bilirubin (mg/dL) Mean ± SD	0.60 ±0.12	0.20 ±0.01	0.12 ±0.01	χ^2= 36.65 [a] **P-value <0.001 HS**	**p1=0.006 HS** **p2<0.001 HS** **p3<0.001 HS**
Albumin (g/dL) Mean ± SD	2.90 ±0.41	3.10 ±0.41	4.00 ±0.31	χ^2= 50.70 [a] **P-value <0.001 HS**	p1=0.110 NS **p2<0.001 HS** **p3<0.001 HS**
INR Mean ± SD	1.30 ±0.31	1.20 ±0.28	1.10 ±0.27	χ^2= 38.63 [a] **P-value <0.001 HS**	p1=0.159 NS **p2<0.001 HS** **p3<0.001 HS**
Hemoglobin (g/dL) Mean ± SD	8.50 ±1.21	11.40 ±2.67	14.20 ±3.28	χ^2= 79.01 [a] **P-value <0.001 HS**	**p1<0.001 HS** **p2<0.001 HS** **p3<0.001 HS**
Platelets (10^3 cell/µL) Mean ± SD	98.00 ±12.12	174.00 ±22.98	256.00 ±28.91	χ^2= 63.40 [a] **P-value <0.001 HS**	**p1<0.001 HS** **p2<0.001 HS** **p3<0.001 HS**

IQR: Interquartile range (difference between 1st and 3rd quartiles)
a: Kruskal-Wallis test; if significant, multiple pairwise comparisons were adjusted by Dunn-Sidak post hoc test.
b: Pearson Chi-square test
NS: Non-significant at p-value ≥0.05 S: Significant at p-value < 0.05
HS: Highly significant at p-value < 0.01
p1:p-value for the difference between LC with EV and LC without EV groups (GI vs. GII)
p2: p-value for the difference between LC with EV and healthy controls (GI vs. GIII)
p3: p-value for the difference between LC without EV and healthy controls groups (GII vs. GII

In addition, it was found that 60% and 28.6% of cirrhotic patients with EV were graded Child B and C respectively, 25.7% had encephalopathy, 68.6% had large varices, and 31.4% with small varices **(Table 2).**

Table (2): Clinical parameters in patients had (LC) with and without (EV)

Clinical parameters	**G I** **LC with EV** **(n= 35)**	**G II** **LC without EV** **(n= 35)**	**Significance test**	**P-value**
PV diameter (mm) Mean ± SD	13.56 ± 1.75	10.17 ± 0.88	*t*= 10.22 [a]	**<0.001** [HS]
Spleen diameter (mm) Median (IQR)	165.00 (27.00)	126.00 (20.00)	*z*= 5.95 [b]	**<0.001** [HS]
Platelets count/spleen diameter ratio (cell/µL)/mm Mean ± SD	607.40 ±38.98	1400.00 ±15.85	*z*= 6.70 [b]	**<0.001** [HS]
Child Score Median (IQR)	8.00 (3.00)	7.00 (3.00)	*z*= 3.92 [b]	**<0.001** [HS]
Child Classification (n (%)) A B C	4 (11.4) 21 (60.0) 10 (28.6)	16 (45.7) 18 (51.4) 1 (2.9)	χ^2= 14.79 [c]	**0.001** [HS]
Ascites (n (%)) None Mild Moderate	7 (20.0) 22 (62.9) 6 (17.1)	25 (71.4) 8 (22.9) 2 (5.7)	χ^2= 18.85 [d]	**<0.001** [HS]
Varices (n (%)) No Small Large	0 (0.0) 11 (31.4) 24 (68.6)	35 (100.0) 0 (0.0) 0 (0.0)	χ^2= 70.00 [c]	**<0.001** [HS]
Encephalopathy (n (%)) No Yes	26 (74.3) 9 (25.7)	35 (100.0) 0 (0.0)	χ^2= 10.33 [d]	**0.002** [HS]
Blood transfusion (n (%)) No Yes	19 (54.3) 16 (45.7)	35 (100.0) 0 (0.0)	χ^2= 20.74 [c]	**<0.001** [HS]
Diabetes Mellitus (DM) (n (%)) No Yes	24 (68.6) 11 (31.4)	27 (77.1) 8 (22.9)	χ^2= 0.65 [c]	0.420 [NS]
Hyper tension (HTN) (n (%)) No Yes	23 (65.7) 12 (34.3)	24 (68.6) 11 (31.4)	χ^2= 0.07 [c]	0.799 [NS]
Smoking (n (%)) No Yes	27 (77.1) 8 (22.9)	28 (80.0) 7 (20.0)	χ^2= 0.09 [c]	0.771 [NS]
Cirrhosis etiology (n (%)) Cryptogenic NASH Budd Chiari Bilharzial HCV	0 (0.0) 0 (0.0) 1 (2.9) 1 (2.9) 33 (94.3)	1 (2.9) 3 (8.6) 0 (0.0) 3 (8.6) 28 (80.0)	χ^2= 5.82 [d]	0.153 [NS]

PV: Portal vein; HCV: Hepatitis C virus; NASH: non-alcoholic steatohepatitis
IQR: Interquartile range (difference between 1st and 3rd quartiles)
a: Student t-test b: Mann-Whitney test
c: Pearson chi-square test d: Fisher's Exact test

2- HO-1 polymorphism (rs2071746) genotypes and alleles distribution among study subjects

The frequency distributions of the different genotypes and alleles for HO-1 polymorphism are shown in **(table 3).**

In LC with EV group, AA genotype was prevalent in 42.9% of patients, AT genotype in 51.4%, and TT genotype in 5.7%.

An allele was predominant (68.6%). The dominant model (AA+AT versus TT) in LC with EV patients group revealed a significant difference (P=0.04) compared to LC without EV and healthy control groups.

3- HO-1 gene polymorphism (rs2071746) and the risk for EV occurrence in LC with EV group

Also, **(Table 3)** showed that the AA genotype was associated with significant increased risk of EV in cirrhotic patients compared to healthy controls (OR= 6.82, 95% CI =1.24 - 37.54; p= 0.018), AT genotype with OR =6.43 (95% CI =1.21 - 34.19; p= 0.019). The A allele was significantly associated with an increased risk of EV (OR= 2.06, 95% CI=1.04 - 4.10; p= 0.038). In addition, it was revealed that the dominant model (AT+AA versus TT) genotype was significantly associated with increased risk of EV in cirrhotic patients compared to healthy controls and cirrhotic patients without EV (OR= 6.60, p= 0.011 and 4.89, P= 0.040 respectively).

Table (3): Genetic analysis of genotypic distribution and allelic frequencies of heme oxygenase-1 (rs2071746) in patients who had liver cirrhosis with and without esophageal varices and healthy controls.

HO-1 polymorphism genotype	LC with EV n= 35	LC with-out EV n= 35	Healthy controls n= 35	Chi-square test ($\chi 2$)	P-value [a]	LC with EV vs. LC without EV		LC with EV vs. Healthy controls		LC without EV vs. Healthy controls	
						OR (95% CI)	P-value	OR (95% CI)	P-value [a]	OR (95% CI)	P-value [a]
Genotypes				6.44	0.169 NS						
AA	15 (42.9)	12 (34.3)	11 (31.4)			5.00 (0.89 - 28.08)	0.073 NS [b]	6.82 (1.24 - 37.54)	**0.018** S	1.36 (0.40 - 4.71)	0.632 NS
AT	18 (51.4)	15 (42.9)	14 (40.0)			4.80 (0.88 - 26.12)	0.076 NS, [b]	6.43 (1.21 - 34.19)	**0.019** S	1.34 (0.41 - 4.36)	0.627 NS
TT	2 (5.7)	8 (22.9)	10 (28.6)			Ref.	-	Ref.	-	Ref.	-
Dominant model [c]	33 (94.3)	27 (77.1)	25 (71.4)	6.42	**0.040** S	4.89 (0.96 - 24.97)	**0.040** S, a	6.60 (1.33 - 32.84)	**0.011** S	1.35 (0.46 - 3.96)	0.584 NS
Recessive model [d]	15 (42.9)	12 (34.3)	11 (31.4)	1.07	0.585 NS	1.44 (0.55 - 3.78)	0.461 NS, [a]	1.64 (0.62 - 4.35)	0.322 NS	1.14 (0.42 - 3.09)	0.799 NS
Alleles				4.59	0.101 NS						
A	48 (68.6)	39 (55.7)	36 (51.4)			1.73 (0.87 - 3.46)	0.117 NS, [a]	2.06 (1.04 - 4.10)	**0.038** S	1.19 (0.61 - 2.31)	0.611 NS
T (Ref)	22 (31.4)	31 (44.3)	34 (48.6)			Ref.	-	Ref.	-	Ref.	-
***P* HWE [e]**	0.180 NS	0.478 NS	0.135 NS								

a: Pearson Chi-square test b:Fisher's Exact test
c: Dominant model: homozygous wild hetero type(AA+AT) vs. homozygous variant TT.
d: Recessive model: homozygous wild AA vs. hetero type + homozygous variant (AT+TT)
e : *P* HWE**,** for testing Hardy-Weinberg equilibrium

4-Relation between the different genotypes and laboratory data among LC with EV patients

We found that AA genotypes were significantly associated with low platelet levels compared to AT & TT genotypes (p= 0.026). However, no statistical significance was detected between the different genotypes as regard age and other laboratory parameters (ALT, AST, albumin, ALP, GGT, total & direct bilirubin, INR, and hemoglobin), **(Table 4).**

Table (4): Statistical analysis of demographics and laboratory parameters according to genotypes AA vs. AT/TT of polymorphism in LC with EV group

Parameters	Heme oxygenase-1 polymorphism in LC with EV group		Significance test	P-value
	AA (n= 15)	AT/TT (n= 20)		
Age (years) Mean ± SD	55.93 ± 8.89	56.65 ± 8.30	*t*= 0.25 [a]	0.808 [NS]
AST (U/L) Mean ± SD	38.00 ±5.12	53.00 ±11.85	*z*= 1.55 [b]	0.121 [NS]
ALT (U/L) Mean ± SD	17.00 ±4.1	21.00 ±4.85	*z*= 0.45 [b]	0.652 [NS]
ALP (U/L) Mean ± SD	85.00 ± 11.12	105.00 ±22.59	*z*= 0.68 [b]	0.494 [NS]
GGT (U/L) Mean ± SD	38.00 ±4.91	52.50 ± 12.84	*z*= 0.55 [b]	0.582 [NS]
Total bilirubin (mg/dL) Mean ± SD	2.40 ±0.11	1.75 ±0.21	*z*= 1.87 [b]	0.062 [NS]
Direct bilirubin (mg/dL) Mean ± SD	0.70 ±01	0.51 ±0.11	*z*= 1.14 [b]	0.256 [NS]
Albumin (g/dL) Mean ± SD	2.77 ± 0.5	2.82 ± 0.60	*t*= 0.23 [a]	0.820 [NS]
INR Mean ± SD	1.30 ±0.21	1.30 ±0.22	*z*= 0.25 [b]	0.801 [NS]
Hemoglobin (g/dL) Mean ± SD	8.50 ±1.81	8.35 ±1.92	*z*= 0.74 [b]	0.462 [NS]
Platelets (10^3 cell/µL) Mean ± SD	89.93 ± 21.94	115.05 ± 4.76	*t*= 2.34 [a]	**0.026** [S]
IQR: Interquartile range (difference between 1st and 3rd quartiles) a: Student t-test b: Mann-Whitney test				

5-Relation between the different genotypes, clinicopathological and demographic data among LC with EV patients

AA genotype was significantly associated with lower platelets count/ spleen diameter ratio than AT+TT patients (p= 0.014) (table 5). Moreover, 86.7 % of AA genotypes and 61.1% of AT genotypes were having large varices, while there were no TT genotypes that had large varices (table 6). On the other hand, no statistically significant difference between the different genotypes regarding other gender and other clinical parameters **(Tables 5 and 6).**

Table (5): Statistical analysis of clinical parameters according to genotypes AA vs. AT/TT of heme oxygenase-1 polymorphism (rs2071746) in LC with EV group

Clinical parameters	Heme oxygenase-1 polymorphism in LC with EV group		Significance test	P-value
	AA (n= 15)	AT + TT (n= 20)		
PV diameter (mm) Mean ± SD	12.85 ± 1.25	14.10 ± 1.91	*t*= 2.32 [a]	**0.027** [S]
Spleen diameter (mm) Median (IQR)	165.0 (45.0)	164.0 (35.5)	*z*= 0.79 [b]	0.432 [NS]
Child Score Mean ± SD	9.07 ± 2.22	7.95 ± 1.50	*t*= 1.78 [a]	0.085 [NS]
Platelets count/ spleen diameter ratio (cell/µL)/mm Mean ± SD	522.97 ± 15.64	715.67 ± 81.83	*t*= 2.60 [a]	**0.014** [S]
PV: Portal vein; HCV: Hepatitis C virus; NASH: non-alcoholic steatohepatitis IQR: Interquartile range (difference between 1st and 3rd quartiles) a: Student t-test b: Mann-Whitney test , NS : Non significant at p-value ≥ 0.05 S : significant at p-value < 0.05				

Table (6): Statistical analysis of demographics and clinical parameters according to genotypes of Heme oxygenase-1 polymorphism (rs2071746) in LC with EV group.

Parameter	Heme oxygenase-1 polymorphism in LC with EV group			Fisher's Exact test	P-value
	AA (n= 15)	AT (n= 18)	TT (n= 2)		
Gender (n (%))					
Male	10 (66.7)	12 (66.7)	1 (50.0)	χ^2= 0.59	1.000 NS
Female	5 (33.3)	6 (33.3)	1 (50.0)		
Child Classification (n (%))					
A	1 (6.7)	2 (11.1)	1 (50.0)	χ^2= 6.46	0.129 NS
B	8 (53.3)	13 (72.2)	0 (0.0)		
C	6 (40.0)	3 (16.7)	1 (50.0)		
Ascites (n (%))					
None					
Mild	2 (13.3)	4 (22.2)	1 (50.0)	χ^2= 3.06	0.588 NS
Moderate	9 (60.0)	12 (66.7)	1 (50.0)		
	4 (26.7)	2 (11.1)	0 (0.0)		
Encephalopathy (n (%))					
No	11 (73.3)	13 (72.2)	2 (100.0)	χ^2= 0.50	1.000 NS
Yes	4 (26.7)	5 (27.8)	0 (0.0)		
Varices (n (%))					
Small	2 (13.3)	7 (38.9)	2 (100.0)	χ^2= 6.31	**0.025** S
Large	13 (86.7)	11 (61.1)	0 (0.0)		
Blood transfusion (n (%))					
No				χ^2= 2.85	1.000 NS
Yes	6 (40.0)	11 (61.1)	2 (100.0)		
	9 (60.0)	7 (38.9)	0 (0.0)		
DM (n (%))					
No	10 (66.7)	12 (66.7)	2 (100.0)	χ^2= 0.68	1.000 NS
Yes	5 (33.3)	6 (33.3)	0 (0.0)		
HTN (n (%))					
No	8 (53.3)	13 (72.2)	2 (100.0)	χ^2= 2.00	0.362 NS
Yes	7 (46.7)	5 (27.8)	0 (0.0)		
Smoking (n (%))					
No	11 (73.3)	14 (77.8)	2 (100.0)	χ^2= 0.52	0.225 NS
Yes	4 (26.7)	4 (22.2)	0 (0.0)		
Cirrhosis etiology (n (%))					
Budd Chiari	0 (0.0)	1 (5.6)	0 (0.0)	χ^2= 4.23	1.000 NS
Bilharzial	0 (0.0)	1 (5.6)	0 (0.0)		
HCV	15 (100.0)	16 (88.9)	2 (100.0)		

HCV: Hepatitis C virus ;
IQR: Interquartile range (difference between 1st and 3rd quartiles)
=%percent within genotype subgroups.
NS: Non significant at p-value ≥ 0.05 HS: Highly significant at p-value < 0.01

DISCUSSION

Gastroesophageal varices were the most common portosystemic collaterals and their rupture is considered as the commonest serious and lethal complication of portal hypertension. 70% of gastrointestinal tract bleeding in cirrhosis is due to the rupture of esophageal varices [13].

Early prediction of esophageal varices development is mandatory for the early management of esophageal varices. Due to its role in inhibiting NO, the most powerful vasodilator substance, HO-1 was suggested as a candidate marker for the prediction of the development of esophageal varices in cirrhotic patients [14]. Therefore, the present study was designed to evaluate the influence of single nucleotide polymorphism (SNP) in the promoter region of HO-1 (rs2071746) in the development of varices in cirrhotic patients.

We found that chronic hepatitis C virus infection (HCV) was prevalent in 94.3% of LC with EV patients and 80% of LC without EV. **Helal *et al.*** [15] revealed that HCV infection remained the main cause of chronic liver diseases with a prevalence rate of 85.7% among cases. On studying HO-1 (rs2071746) polymorphism, the present study showed that the AA and AT genotypes were associated with a significantly increased risk of EV in cirrhotic patients compared to healthy controls (OR= 6.82 and 6.43, p= 0.018 and 0.019 respectively). The A allele was significantly associated with an increased risk of EV (OR= 2.06, p= 0.038). Also, the dominant model (AT+AA versus TT) was significantly associated with increased risk of EV in cirrhotic patients compared to healthy controls and cirrhotic patients without EV (OR= 6.60 and 4.89, p= 0.011 and 0.040 respectively). These results were in agreement with **Silva**[14] who found that there was a significant association between AA genotypes of (rs2071746) promoter polymorphism and the presence of EV cases among cirrhotic patients. They reported that the A allele was associated with the high activity of HO-1.

Also, **Ying *et al.*** [16], demonstrated that both AA genotype and A allele were risk factors for the development of EV in cirrhotic patients. Also, **Ying *et al.*** [16] documented that, the AA genotype of HO-1 (rs2071746) polymorphism was significantly associated with an increased risk of EV occurrence (AA vs. AT+TT: OR = 1.19, 95% CI = 1.02–1.43; $p < 0.003$). Interestingly, **Buis *et al.*** [17], reported that patients having the AA genotypes of HO-1 (rs2071746) polymorphism were found to be associated with a higher HO-1 gene promoter activity than the TT genotypes (P =0.004) during liver transplantation and give a better graft survival rate after liver transplantation as upregulation of HO-1 catalyzes the oxidative detoxification of excess heme so has been shown to protect livers from ischemia/reperfusion injury and to improve graft survival.

On the other hand, **Lee *et al.*** [11], demonstrated that patients with TT genotype were significantly more liable to develop diabetic nephropathy & albuminuria than those carrying A allele with an odds ratio of 1.6 as T allele was associated with low expression of HO-1 as a potent antioxidant, and this can explain the more susceptibility of developing vascular complications in type 2 diabetic cases carrying T allele. Also, **Chinedu *et al.*** [18], suggested that the development of renal complications in Brazilian sickle cell anemia patients was significantly higher in TT patients (p = 0.019), as there was an association between the T allele and the reduction of the HO-1 gene expression which resulted in significantly increasing inflammatory markers and vaso-occlusion. Further studies correlating levels of HO-1 and rs2071746 polymorphism in different diseases and evaluating their effect are recommended. Besides, on studying the correlation between HO-1 (rs2071746) genotypes and different clinical and laboratory characteristics, it was found AA genotypes were significantly associated with lower platelet levels vs AT+TT genotypes (P= 0.026) and lower platelets count/ spleen diameter ratio than AT+TT patients (p-value= 0.014). Moreover, 86.7 % of patients with the AA genotype significantly had large varices (p-value= 0.025). This shows that rs2071746 polymorphism could be used as a potential predictor and early diagnostic marker of EV development.

CONCLUSION

The A allele of HO-1 413A>T promoter polymorphism (rs2071746) is significantly associated with the development of esophageal varices along with its course progression. It could be used as a potential predictor and early diagnostic marker of EV development. More studies based on larger sample sizes and more ethnic groups are still needed in the future.

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