Diagnosis of cirrhosis in patients with chronic hepatitis C genotype 4: Role of ABCB11 genotype polymorphism and plasma bile acid levels

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ABSTRACT

Background/Aims: *Chronic hepatitis C (CHC)-related mortality generally results from cirrhosis and subsequent complications. We aimed to investigate the potential role of plasma bile acid levels and ABCB11 1331T>C (V444A, rs2287622) (ATP-binding cassette subfamily B, member 11) gene polymorphism in fibrosis prediction in CHC genotype 4 patients.*

Materials and Methods: *This case control study included 85 healthy control and the following 225 subjects: 170 adult patients infected with hepatitis C virus (HCV) and categorized into three groups according to liver biopsy; no fibrosis group (F0) (n=33), early fibrosis group (F1-F2) (n=61), and advanced fibrosis group (F3-F4) (n=76). Fasting bile acid levels, hepatitis C virus (HCV) genotyping, and ABCB11 1331T>C gene polymorphism were assessed.*

Results: *The frequency of the variant homozygote genotype CC in advanced fibrosis was significantly higher than that in early fibrosis (48.7% vs. 36.1%) (odd ratio, OR =2.58; 95% confidence interval, CI=1.07-6.20; p=0.03). C allele was significantly represented in advanced fibrosis (65.8%) compared with that in early fibrosis (51.6%) (OR=1.80, 95% CI=1.10-2.93, p=0.01). A significant elevation of plasma bile acid levels in advanced fibrosis was observed compared with those in early fibrosis (p≤0.001). Receiver operating characteristic curve for plasma bile acid levels at cutoff value of 75.5 µmol/L had a 59% specificity and 97.4% sensitivity as a predictor of advanced hepatic fibrosis (AUROC=0.78%).*

Conclusion: *We concluded that Egyptian patients having chronic hepatitis C genotype 4 with CC genotype of ABCB11 SNP 1331T>C and high plasma bile acid levels at cutoff value of 75.5 µmol/L were associated with advanced hepatic fibrosis.*

Keywords: *CHC, ABCB11 gene, bile acid, cirrhosis*

INTRODUCTION

Hepatitis C virus (HCV) infection is considered to be the leading cause of chronic liver disease worldwide (1). HCV infection is often asymptomatic but can progress to liver fibrosis and eventually to cirrhosis. Mortality associated with chronic hepatitis C (CHC) essentially results from progression to cirrhosis and its related complications. HCV may be involved in fibrogenesis directly by stimulation of hepatic stellate cells or indirectly by triggering oxidative stress and infected hepatocyte apoptosis (2). Identification of the stage of liver fibrosis or detection of liver cirrhosis in patients with CHC is essential because of its prognostic importance and role in patient management (3). Liver biopsy was proven to be the gold-standard tool to grade inflammation and stage fibrosis. However, its limitations became very evident. Thus, the search for new noninvasive approaches has been initiated (4). In normal conditions, ATP-binding cassette, subfamily B, member11 (*ABCB11*) and other hepatobiliary proteins (i.e., ABCG5/8- and ABCB4-transporting sterols and phospholipids into bile, respectively) maintain appropriate concentrations of biliary components. Functional inhibition of bile salt export pump (BSEP) leads to a decreased bile salt excretion and a reduced flow of bile, which is

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called cholestasis. Mutation in the canalicular transporter genes, particularly in the *ABCB11* gene, lead to functional disorders, leading to inherited cholestasis or increasing vulnerability to acquired cholestasis (5). As many genes have proved to control the serum bile acid levels and bile acids trigger inflammatory process in the liver that stimulates hepatic stellate cells and lead to induction of fibrogenesis, it is proposed that polymorphisms of genes that regulate bile acid levels may affect the progression of liver disease (6-8).

On the basis of the existing knowledge, a few studies have worked on the potential role of plasma bile acid levels and polymorphism of *ABCB11* SNP 1331T>C (V444A, rs2287622) gene on fibrosis prediction in CHC; on the other hand, no data are available about this variation in the Egyptian population. Therefore, the aim of the current study was to investigate the potential role of plasma bile acid levels and *ABCB11* SNP 1331T>C (V444A, rs2287622) gene polymorphism on fibrosis prediction in CHC genotype 4 patients.

MATERIALS AND METHODS

Of the 225 Egyptian individuals included in this study, 170 were adult HCV-infected patients (range, 21-58 y; 98 males and 72 females) and 85 were age-, sex-, and ethnically matched control healthy volunteers (range, 19-53 y; 48 males and 37 females). The patients were recruited between June 2013 and April 2014 from the Tropical Medicine Department, Mansoura University Hospitals, Dakahlya, Egypt. Exclusion criteria comprised patients co-infected with human immunodeficiency virus (HIV) or hepatitis B virus (HBV) (HBs Ag, HBV core antibodies), patients with positive anti-HCV antibodies and negative HCV-polymerase chain reaction (PCR) in the serum, patients with previous or current history of decompensated conditions (hepatic encephalopathy and variceal bleeding), other causes of chronic or metabolic liver diseases (such as autoimmune, Wilson disease, and Gaucher's disease), and morbid obesity (body mass index, BMI <40 kg/m^2), and patients with HCV genotypes other than genotype 4. Chronic HCV infection was diagnosed by detection of an anti-HCV antibody by enzyme-linked immunosorbent assay and confirmed with real-time polymerase chain reaction. All patients underwent routine liver function tests, complete blood count, HCV genotyping, and abdominal ultrasound.

The patients were divided into the following three groups according to the pathological examination of their liver biopsy: HCV-infected patients without liver fibrosis (F0) (n=33), HCV-infected patients with early fibrosis (F1-F2) (n=61), and HCV-infected patients with advanced fibrosis (F3-F4) (n=76).

All patients and control subjects of the study signed a written informed consent. The Ethics Committee of Mansoura University School of Medicine approved the study.

Bile acid quantification

The assessment of fasting bile acid levels from 100 µL plasma, which were diluted with 100 µL saline using bile acid assay kit (Sigma-Aldrich, USA, Cat no. #MAK309), *provided a convenient* fluorimetric means to measure total bile acids in biological samples. 3α-Hydroxysteroid dehydrogenase reacts with all 12 bile acids, converting NAD to NADH and reducing a probe to a highly fluorescent product. Fluorescence intensity (λ_{ex}=530 nm; λ_{em}=585 nm) resulting from this assay is linear to the bile acid concentration in the sample. Blood samples were withdrawn from the patients at the same session of liver biopsy.

HCV genotyping

RNA was extracted using QIAamp Viral RNA Mini kit (Qiagen, Valencia, CA). Pyrosequencing technology for real-time was performed using the PyroMark Q24 software (Qiagen, Valencia, CA). Sequence-based detection and quantification of sequence variants and epigenetic methylation were also conducted. PyroMark Q24 is seamless for the analysis of CpG methylation, single-nucleotide polymorphisms (SNPs), insertion/deletions, short tandem repeats, and variable gene copy number, microbial identification, and resistance typing. Primers and dispensation order were performed as described by Elahi et al. (9).

DNA isolation and genotyping

DNA extraction was performed using the Gene JET Whole Blood Genomic DNA Purification Mini kit (Thermo Scientific, USA, Cat. no #K0781) according to the manufacturer's instructions. rs2287622 is an SNP in the ATP-binding cassette, subfamily B (MDR/TAP), member 11 *ABCB11* gene. The molecular meaning of this missense variant is amino acid substitution. The more common (T) allele encodes a Val, and the rarer (C) allele encodes a Ala; this SNP is also known as V444A or c.1331T>C. The minor allele frequency of the variation in global population is 0.4113/2060 according to the db SNP.

For genotyping of the SNP *ABCB11* 1331>C (V444A, rs2287622), the primers used included 5′-CACACAGA-

CACCGAGTATCAACACA-3′ as F primer and 5′-CAGGACAGTCTCAATGTATGCTACACCT-3′ as R primer. Amplification was carried out using Hot Start Maxima Taq DNA Polymerase (Thermo Scientific). The initial denaturation temperature was 95°C for 4 min followed by cycling, including denaturation at 95°C for 30 s, annealing at 58.0°C for 30 s, and extension at 72°C for 60 s, for 30 cycles, and final extension at 72°C for 5 min. A PCR product of 333 bp was obtained and digested with *Hae*III restriction enzyme. The C allele was digested to produce bands of 206 and 127 bp if positive samples. While the T allele was not digested if positive samples.

Liver biopsy

Liver biopsy (transcostal or subcostal) was performed by expert hepatologist from the right liver lobe using a 16-gauge needle under ultrasound guidance at a minimum of 1.5-cm-length core or by encompassing 10 portal areas in minimum, which is suitable for interpretation. Routine processing included fixation in neutral-buffered formalin, paraffin embedding, routine preparation of hematoxylin and eosin-stained slides, Prussian blue, and Masson trichrome (for collagen I); METAVIR scoring system was applied (10). The following are the fibrosis stages (F0-F4): F0, non-fibrosis; F1, portal tract expansion by fibrosis (Figure 1); F2, <50% bridging fibrosis; F3, >50% bridging fibrosis (Figure 2); and F4, established cirrhosis.

Statistical analysis

Data were analyzed using the Statistical package for social science computer program version 20 (IBM Corp.; Armonk, NY, USA). The normality of the distribution was tested using Kolmogrov-Smirnov test. Numerical variables were expressed as mean, standard deviation, median, minimum, and maximum. For comparing two groups, *t*-test (for parametric) or Mann-Whitney test (for non-parametric) were used. For comparing >2 groups, analysis of variance (for parametric) or Kruskal-Wallis test (for non-parametric) were used. Comparison among groups was performed using chi-square or Fisher exact tests. The SNP was tested for Hardy-Weinberg equilibrium. Genotype distributions between patients and controls, odds ratio (OR) and 95% confidence interval (CI) were calculated to detect risk ratio. The cutoff point was selected as the point with the highest sensitivity and specificity rates using receiver operator characteristics (ROC) curve. Logistic regression analysis was used for the prediction of risk factors for late fibrosis. $p \leq 0.05$ was considered statistically significant in all analyses.

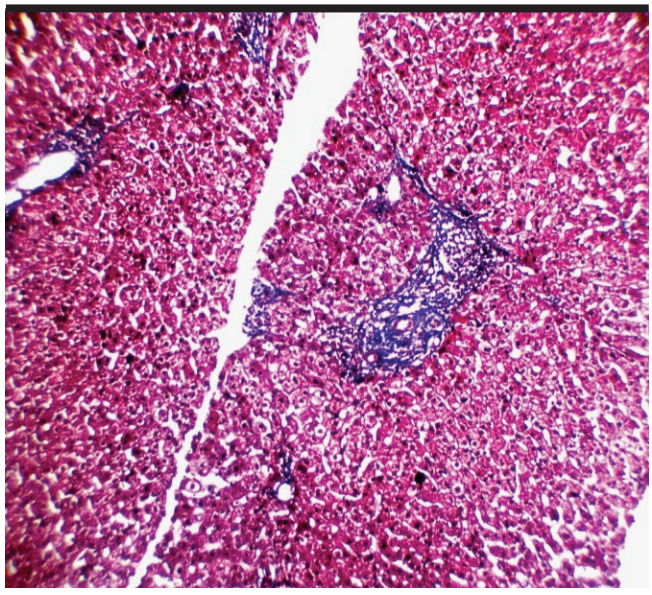

Figure 1. F1: Liver tissue with portal tracts expansion by fibrosis (MT stain, 100×)

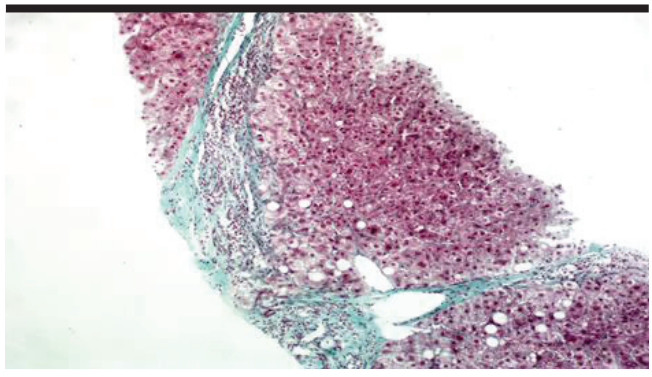

Figure 2. F3: Liver tissue with portal tracts expansion by fibrosis and bridging fibrosis (MT stain, 100×)

RESULTS

Demographic data and characteristics of the studied subjects

Tables 1 and 2 summarize the baseline characteristics of patients. A total of 170 patients with CHC and 85 healthy controls were included in this study. No age-based evident differences were observed between males and females. There was a predominance of males in the group of patients with CHC. The control group was largely balanced. Age and sex of HCV group patients were matched with those of the control.

Table 1. Comparison of demographic, anthropometric, and laboratory data in control, F0, and fibrosis groups

		Control (N=85)	F0 (N=33)	Fibrosis (N=137)	p1	p2
Age (years) mean (SD)		36.5 (10.1)	28.9 (4.9)	41.3 (6)	0.067	≤0.001
Sex N (%)	M	48 (56.5)	8 (24.2)	90 (65.7)	0.858	≤0.001
	F	37 (43.5)	25 (75.8)	47 (34.3)		
BMI (kg/m^2) Mean (SD)		26.8 (±3.5)	26.7 (±3.5)	28.1 (±3.8)	0.931	0.023
ALT (IU/L) Median (min-max)		37 (23-50)	40 (26-77)	57 (24-188)	0.018	≤0.001
AST (IU/L) Median (min-max)		35 (22-51)	38 (24-66)	54 (24-100)	0.070	≤0.001
Total Bilirubin (mg/dL) Median (min-max)		0.8 (0.5-1)	0.7 (0.3-1)	1.2 (.4-4.6)	0.176	0.041
S. albumin (g/dL) Median (min-max)		4.3 (3.2-5.2)	4.5 (3.9-5.3)	3.6 (3.3-5.1)	0.276	0.031
INR Median (min-max)		1 (0-1.2)	0.9 (0.8-1.2)	1.2 (.5-1.3)	0.129	0.021
ALP (IU/L) Median (min-max)		48 (22- 90)	85 (51-182)	130 (40-190)	≤0.001	≤0.001
Bile acid levels (μmol/L) Median (min-max)		13 (4.4-22)	23 (15-41)	78 (10-110)	≤0.001	≤0.001

p1: significance between control and F0; p2: significance between F0 and fibrosis; SD: standard deviation; BMI: body mass index; ALT: alanine transaminase; AST: aspartate transaminase; INR: international normalized ratio; ALP: alkaline phosphatase

Table 2. Comparison of demographic, anthropometric, and laboratory data in CHC patients with early and advanced fibrosis

		Early fibrosis (F1&F2) (N=61)	Advanced fibrosis (F3&F4) (N=76)	p
Age (years) Mean (SD)		38.4 (6.6)	43.6 (4.2)	≤0.001
Sex No (%)	M	38 (62.3)	52 (68.4)	0.453
	F	23 (37.7)	24 (31.6)	
BMI (kg/m^2) Mean (SD)		27.2 (±3.3)	28.7 (±4.1)	0.062
ALT (IU/L) Median (min-max)		44 (24-144)	60 (28-188)	0.002
AST (IU/L) Median (min-max)		50 (24-98)	54.5 (30-100)	0.087
Total Bilirubin (mg/dL) Median (min-max)		.9 (.4-1.3)	1.2 (0.7-4.6)	0.041
S. albumin (g/dL) Median (min-max)		4.2 (3.4-5.1)	3.4 (3.3-4.9)	0.031
INR Median (min-max)		1 (.5-1.3)	1.2 (1-1.3)	0.183
ALP (IU/L) Median (min-max)		100 (40-156)	145 (90-190)	≤0.001
Bile acid levels (μmol/L) Median (min-max)		45 (10-93)	89 (68-110)	≤0.001

p: significance between early and advanced fibrosis; SD: standard deviation; BMI: body mass index; ALT: alanine transaminase; AST: aspartat transaminase; INR: international normalized ratio; ALP: alkaline phosphatase.

Distribution of ABCB11 SNP 1331T>C (V444A, rs2287622) genotypes in patients with CHC and control subjects

Genotyping of *ABCB11* SNP 1331T>C was performed in 170 patients with CHC and was compared with 85 healthy control in terms of allele and genotype frequencies (Table 3). The frequency of the homozygote genotype CC in HCV patients (38.8%) was significantly higher than that in the control group (16.5%) (OR=3.83, 95% CI=1.87-7.82, p≤0.0001). Similarly, the occurrence of C allele was significantly over-represented in HCV patients (55.3%) than that in the control patients (35.3%) (OR=2.26, 95% CI=1.55-3.31, p≤0.0001). Moreover, the frequency of combined TC+CC genotypes in HCV group (71.8%) was significantly higher than that in the control group (54.1%) (OR=2.15, 95% CI=1.25-3.70, p=0.001).

Distribution of ABCB11 SNP 1331T>C (V444A, rs2287622) in different fibrosis stages of CHC patients

The *ABCB11* SNP 1331T>C genotype distribution throughout the different stages of fibrosis is demonstrated in Table 3. The frequency of the homozygote genotype CC of *ABCB11* in the fibrosis group was significantly higher than that in the F0 group (43.1% vs. 21.2%, respectively) (OR=3.83, 95% CI=1.41-10.34, p=0.008). Similarly, the occurrence of C allele of *ABCB11* SNP 1331T>C was significantly over-represented in the fibrosis group (59.5%) than that in the F0 group (37.9%) (OR=2.40, 95% CI=1.38-4.18, p=0.002). Moreover, the

Table 3. Comparison of genotypes and alleles of rs2287622 polymorphism of *ABCB 11* gene in the control and HCV patients according to fibrosis stage

		Control (N=85)	HCV (N=170)	F0 (N=33)	Fibrosis (N=137)	Early fibrosis (F1&F2) (N=61)	Advanced fibrosis (F3&F4) (N=76)
Genotypes	TT	39 (45.9)	48 (28.2)	15 (45.5)	33 (24.1)	20 (32.8)	13 (17.1)
	TC	32 (37.6)	56 (32.9)	11 (33.3)	45 (32.8)	19 (31.1)	26 (34.2)
	CC	14 (16.5)	66 (38.8)	7 (21.2)	59 (43.1)	22 (36.1)	37 (48.7)
HW p		0.105	<0.001	0.094	0.0002	0.003	0.036
C containing genotypes	TC+CC	46 (54.1)	122 (71.8)	18 (54.5)	104 (75.9)	43 (70.5)	63 (82.9)
Alleles	T	110 (64.7)	152 (44.7)	41 (62.1)	11q (40.5)	59 (48.4)	52 (34.2)
	C	60 (35.3)	188 (55.3)	25 (37.9)	163 (59.5)	63 (51.6)	100 (65.8)
				Statistical comparison			
		p1	OR (95% CI)	p2	OR (95% CI)	p3	OR (95% CI)
Genotypes	TT		1		1		1
	TC	0.255	1.422 (0.776-2.606)	0.176	1.860 (0.757-4.566)		0.111 2.105 (0.843-5.257)
	CC	≤0.001	3.830 (1.874-7.829)	0.008	3.831 (1.419-10.343)	0.033	2.587 (1.078-6.208)
C containing genotypes	TC+CC	0.005	2.155 (1.254-3.704)	0.016	2.626 (1.193-5.782)	0.035	2.364 (1.061-5.268)
Alleles	T		1				1
	C	≤0.001	2.268 (1.550-3.317)	0.002	2.408 (1.386-4.186)	0.018	1.801 (1.105-2.935)

TT is the reference genotype; HW p: Hardy -Weinberg equation; OR: Odd's ratio; CI: Confidence interval; p1: significance between control & HCV; p2: significance between F0 & fibrosis groups; p3: significance between early and advanced fibrosis.

frequency of the combined TC+CC genotypes of *ABCB11* in the fibrosis group was significantly higher than that in the F0 group (75.9% vs. 54.5%) (OR=2.62, 95% CI=1.19-5.78, p=0.01).

According to the fibrosis stage, the frequency of the homozygote genotype CC of *ABCB11* SNP 1331T>C in the advanced fibrosis group was significantly higher than that in the early fibrosis group (48.7% vs. 36.1%, respectively) (OR=2.58, 95% CI=1.07-6.20, p=0.03). C allele of *ABCB11* SNP 1331T>C was significantly over-represented in the advanced fibrosis group (65.8%) than that in the early fibrosis group (51.6%) (OR=1.80, 95% CI=1.10-2.93, p=0.018). Moreover, the frequency of combined TC+CC genotypes of *ABCB11* SNP 1331T>C in the advanced fibrosis group (82.9%) was significantly than that in the early fibrosis group (70.5%) (OR=2.36, 95% CI=1.06-5.26, p=0.03).

Levels of bile acids in patients with CHC with different ABCB11 SNP 1331T>C (V444A, rs2287622) genotypes and fibrosis stages

We quantified bile acid levels according to the fibrosis stages (Table 1, 2). Median bile acid levels were 23 µmol/L for F0 group, 45 µmol/L for early fibrosis, and 89 *µmol*/L for advanced fibrosis (p≤0.001). There was a significant elevation of bile acid levels in advanced fibrosis group compared with those in F0 and early fibrosis groups (p<0.001).

The bile acids were quantified to determine whether there was a significant difference in their levels accord-

Table 4. Fasting bile acid values in different genotypes of 1331T V444A (rs2287622) polymorphism of ABCB 11 gene in different studied groups

	Genotyping			
	TT(N=48)	TC(N=56)	CC(N=66)	p
F0 (N=33)	20 (15-34)	23 (15-41)	28 (15-36)	0.373
Fibrotic (F1-F4) (N=137)	65 (10-100)[a, b]	77 (26-100)[a, c]	89 (24-110)[b, c]	0.001
p1	≤0.001	≤0.001	≤0.001	
Early fibrosis (F1 &F2) (N=61)	33 (10-77)[b]	55 (26-76)	63 (24-93)[b]	0.011
Advanced fibrosis (F3&F4) (N=76)	80 (76-100)[b]	86.5 (70-100)[c]	90 (68-110)[b,c]	0.011
p2	≤0.001	≤0.001	≤0.001	

Data expressed either as median (min-max); P: comparison between 3 genotypes in each group; acomparison between TT and TC; bcomparison between TT and CC; ccomparison between TC and CC; p1: comparison between F0 and fibrosis in terms of each genotype; p2: comparison between early and advanced fibrosis in terms of each genotype

Table 5. Univariate and multivariate analyses for the prediction of fibrosis in early and advanced fibrosis

	Advanced versus early fibrosis			
	UNIVARIATE		MULTIVARIATE	
	p	OR (95%)	p	OR (95%)
Age (years)	≤0.001	1.195 (1.107-1.289)	0.405	0.891 (0.679-1.169)
Sex	0.453	0.763 (0.375-1.549)		
BMI (kg/m^2)	0.146	1.064 (0.979-1.156)		
ALT (IU/L)	0.023	1.013 (1.002-1.025)	0.487	1.020 (0.965-1.078)
AST (IU/L)	0.164	1.013 (.995-1.033)		
S. bilirubin (mg/dL)	≤0.001	1.337 (1.095-2.233)	0.096	1.804 (0.288-6.486)
S. albumin (g/dL)	≤0.001	0.122 (0.11-0.82)	0.869	1.535 (0.009-12.990)
INR	≤0.001	1.265 (1.028-2.198)	0.023	4.291 (2.781-7.061)
ALP (IU/L)	≤0.001	1.089 (1.059-1.121)	0.074	1.050 (0.995-1.107)
Plasma bile acid levels (μmol/L)	≤0.001	1.158 (1.093-1.227)	0.010	1.280 (1.062-1.544)
Genotyping (CC+CT/TT)	0.035	2.364 (1.061-5.268)	0.042	3.404 (1.571-16.119)

OR: odds ratio; CI: confidence interval; BMI: body mass index; ALT: alanine transaminase; AST: aspartate transaminase; INR: international normalized ratio; ALP: alkaline phosphatase

ing to different *ABCB11* 1331T>C genotypes in HCV patients (Table 4). When HCV patients were categorized into fibrotic and non-fibrotic groups, there was a significant difference among different genotypes of *ABCB11* 1331T>C (TT, TC, and CC) in fibrotic group in terms of fasting plasma bile acid levels (p≤0.001). The median bile acid levels for CC genotype of *ABCB11* SNP 1331T>C were 89 μmol/L. Also, there was a significant elevation of plasma bile acid levels in fibrotic compared with those in F0 groups in terms of different genotypes (p≤0.001). When fibrotic group was categorized into early and advanced groups, there was a significant difference among different genotypes of *ABCB11* SNP 1331T>C (TT, TC, and CC) in each group in terms of fasting plasma bile acid levels (p=0.001). The median bile acid levels for CC genotype of *ABCB11* SNP 1331T>C was 63 μmol/L in early

Table 6. AUC and performance criteria of total bile acids levels for discrimination between early and advanced fibrosis

AUC (95%CI)	p	Cutoff value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
0.780 (0.695-0.866)	≤0.001	75.5	97.4	59	74.7	94	80.3

AUC: area under the curve; CI: confidence interval; PPV: positive predictive value; NPV: negative predictive value

fibrosis and 90 µmol/L in advanced fibrosis. Also, there was a significant elevation of plasma bile acid levels in advanced fibrosis when compared with those in early the fibrosis groups in terms of different genotypes (p≤0.001).

Table 5 shows logistic regression analysis that was conducted for the prediction of progression of fibrosis from early to advanced using age, sex, BMI, *ABCB11* SNP 1331T>C genotypes as covariates, and alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin, albumin, international normalized ration (INR), alkaline phosphatase (ALP), and plasma bile acid levels. Higher age, ALT, bilirubin, INR, and plasma bile acid levels, lower albumin concentration and (CC+CT) genotype numbers were associated with the risk of fibrosis progression in univariate analysis. In multivariate analysis, only higher INR, plasma bile acid levels, and (CC+CT) genotype numbers of *ABCB11* SNP 1331T>C (OR=4.291, 1.280, and 3.404, respectively) were considered as risk factors for fibrosis progression from early to advanced fibrosis.

The ROC curves for plasma bile acid levels as a predictor of hepatic fibrosis severity in early fibrosis (F1-F2) and advanced fibrosis (F3-F4) groups were generated (Figure 3), and the plasma bile acid levels were considered to be a good predictor of hepatic fibrosis severity (AUROC=0.78). A cutoff value of 75.5 *µmol/*L had 59% specificity and 97.4% sensitivity rates for the prediction of hepatic fibrosis severity. Positive predictive value, negative predictive value, and accuracy were 74.7%, 94%, and 80.3%, respectively (Table 6).

DISCUSSION

The natural history of hepatic fibrosis progression in patients with CHC is considred tobe controversial (11). Several factors such as sex, age, age at infection, duration of the disease, HIV or HBV co-infection, and influence of alcohol consumption cannot accurately predict the individual risk of cirrhosis development in patients with CHC (12, 13). Additionally, genetic factors, such as cirrhosis risk score based on seven genetic variants (seven SNPs) at cutoff value ≥0.8, that affect fibrogenesis and are believed to clarify CHC prognosis are considered to be the predictors of higher fibrosis progression rate (14).

This study evaluated the role of *ABCB11* SNP 1331T>C (V444A, rs2287622) gene polymorphism and fasting plasma bile acid levels in the prediction of hepatic fibrosis in CHC genotype 4 patients.

The present study found that the occurrence of the variant homozygote genotype CC and C allele of *ABCB11* SNP 1331T>C in the fibrosis group was higher than that in the F0 group. Moreover, the occurrence of the homozygote genotype CC and C allele of *ABCB11* SNP 1331T>C in the advanced fibrosis group was higher than that in the early fibrosis group. Furthermore, increased levels of bile acids were observed in HCV patients compared with that in the healthy control and in the patients with advanced fibrosis compared with that in the early fibrotic and non-fibrotic patients (F0). These findings were consistent with those of a previous studies that found an association between *ABCB11* SNP 1331T>C polymorphism and development of cirrhosis in patients with CHC with a carrier risk of >2-fold (15). Stieger and Geier (16) reported that a common variant of *ABCB11* 1331T>C is a recognized factor that is susceptible to acquired cholestasis, and there is an evidence suggesting that this variant also affects the response to treatment and CHC progression. Shlomai et al. (3) classified the patients into two groups (non-severe and severe liver fibrosis/cirrhosis) and reported that serum bile acid levels were significantly elevated in patients with CHC and advanced liver fibrosis as proved by liver biopsy compared with those in HCV patients with non-severe fibrosis (p=0.0021). These findings could be explained by bile acid levels inside the liver that are controlled by the action of BSEP, which is the protein product of *ABCB11* gene and is located at the hepatocyte canalicular membrane. The accumulation of bile acids in the hepatocytes in different patterns of cholestatic liver diseases, which contributes to stimulation of hepatocyte-derived monocyte chemotaxis protein-1(MCP-1), results in mobilization of hepatic stem cells (HSCs); thus, may be considered as an early step in hepatic fibrogenesis (8).

The present study found that there is a significant elevation in plasma bile acid levels in patients with CC genotype of *ABCB11* SNP 1331T>C compared with those in other genotypes (TC, TT) in each of the control, F0, and

fibrotic groups (p<0.001). From these results, we understand that patients with CHC with higher bile acid levels and with CC genotype or C allele are more susceptible to progression of liver fibrosis. This finding correspond to the observations found by Iwata et al. (15) who observed that serum bile acid levels were elevated in HCV patients with CC genotype more than those in other patients with TC and TT genotypes, but this elevation still lies within the normal scope as that of the general population and was not statistically significant (p=0.20 for 1331CC compared with TT) and is possibly related to cirrhosis as cirrhosis rate among patients with the *ABCB11* SNP 1331T>C (23.6%) is higher than that in the other genotypes (1331TC: 12.8% and 1331TT: 10.7%, respectively). *ABCB11* gene polymorphism and BSEP dysfunction were also involved in a variety of cholestatic disorders, including intrahepatic cholestasis of pregnancy (ICP), which manifested in the last trimester of pregnancy, and characterized by raised serum bile acid levels (17). Allelic analysis for C vs. T (OR=1.7, p<0.001). Additionally, the CC homozygotes were more likely to have ICP than the TT homozygotes (OR=2.8, p<0.0001) (18). In contraceptive-induced cholestasis (CIC), a pathogenic relevance of the *ABCB11* SNP 1331T>C polymorphism was noted. There was a highly significant association between the C allele at position 1331 of *ABCB11* and cholestasis (19).

The logistic regression analysis that was conducted for the prediction of progression of revealed that in multivariate analysis, INR, plasma bile acid levels, and CC+CT genotypes of *ABCB11* SNP 1331T>C were considered to be the risk factors for fibrosis prediction. In the study conducted by Iwata et al. (15), logistic regression was conducted between non-cirrhotic (F0,1,2&3) and cirrhotic (F4) groups, and they reported that *ABCB11* SNP 1331T>C CC genotype was associated with progression to cirrhosis and was independent of other risk factors such as sex, age, BMI, duration of the disease, and HCV genotype.

The ROC curve for plasma bile acid levels at cutoff value of 75.5 µmol/L had a 59% specificity and 97.4% sensitivity for the prediction of advanced liver fibrosis (AUROC=0.78%). In a study carried out by Shlomai et al. (3), ROC curve showed that serum bile levels acid was not an excellent predictor for progression of liver fibrosis from non-severe to severe fibrosis (AUROC=67%, sensitivity=64%, specificity=61%). These values were improved after integration of serum bile acid levels in a bile acid-based model, including serum bile acid levels, age, BMI, cholesterol levels, and blood glucose (AUROC=83%, sensitivity=93%, specificity=65%).

The genetics of *ABCB11* polymorphisms with fibrosis progression in patients with CHC indicates that patients who have higher bile acid levels are a good target for interference with bile acid levels-lowering drugs.

There are some limitations to this study. Othe study sample size was small, and large-scale studies are required to estimate the validity of *ABCB11* SNP 1331T>C CC genotype and high plasma bile acid levels in various clinical settings of CHC.

Our study concluded that among Egyptian patients with CHC genotype 4, the CC genotype of *ABCB11* SNP 1331T>C that was present in 38.3% of patients with CHC is a high-risk allele and high plasma bile acid levels at cutoff value of 75.5 µmol/L were associated with progression to advanced liver fibrosis.

Ethics Committee Approval: Ethics committee approval was received for this study from the Institutional Review Board of Mansoura School of Medicine (MS/261).

Informed Consent: Written informed consent was obtained from all the patients who participated in this study.

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