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Clinical Significance of HLA DPB1 (SNPrs3116996 & SNPRs2071025) Gene Polymorphism in Liver Cirrhosis Development among Egyptian Patients with Chronic HCV Infection

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ABSTRACT

Background: Chronic liver disease, which can lead to cirrhosis and hepatocellular carcinoma (HCC), is mostly caused by hepatitis C. **Objective:** We aimed to clarify the association between HLADPB1 expression and chronic HCV infection in Egyptian patients.

Patients and methods: 85 adult participants were enrolled in this study. They were divided into three groups: **Group 1** included 20 cirrhotic patients with HCV, **group 2** included 20 chronic HCV patients and **group 3** included 45 healthy controls. HLADPB1SNPrs3116996 and HLADPB1 SNPRs2071025 polymorphism were assessed.

Results: There was significant higher frequency of **HLADPB1SNPrs3116996** genotype **TA** in cirrhotic HCV patients in comparison with chronic HCV patients and healthy controls **(40%, 15%** and **4.4 %** respectively; **p=0.002**). Also, cirrhotic HCV group had significantly higher frequency of **A** allele when compared to chronic HCV group and healthy controls (**20.0%**, **7.5%** and **2.2%** respectively; **p<0.001**). **HLADPB1 (SNPRs2071025)** genotypes **AG** in patients with cirrhotic HCV, chronic HCV patients and healthy control were **45%**, **25% and 8.9%** respectively with significant P-value **(0.001).** In addition, there was significantly higher allele **G** frequency among the cirrhotic HCV patients **(22.5%)** followed by chronic HCV patients **(17.5%)** compared to controls **(4.4%)** with significant p-value (**0.002)**.

Conclusion: HLADPB1SNPrs3116996 genotype **TA** and **HLADPB1 SNPRs2071025** genotype **AG** carry risk of liver cirrhosis development in patients with chronic HCV infection and deterioration of clinical and biochemical parameters.

Keywords: Hepatitis C virus, Liver cirrhosis, HLA-DPB1 SNP, Genotype, Polymorphism.

INTRODUCTION

The illness hepatitis C has a major worldwide effect [1]. The prevalence is more than 10% in Egypt. One of the main causes of chronic liver disease, which progresses to progressive hepatic fibrosis and eventually cirrhosis and HCC, is chronic HCV infection [2]. The development of oral Direct-Acting Antivirals (DAAs), which directly block the HCV replication cycle and target three key HCV genome regions (NS3/4A protease, NS5A, and NS5B RNA-dependent polymerase) led to a significant advancement in HCV therapy [3]. **Raouf *et al.*** [4] have documented the impact of numerous variables on the onset and course of disease, including genetic variants, host factors (age, sex, environment, and related medical conditions) and viral factors like viral genotype and viral load.

The host's immunity can have a major effect on the outcome of an HCV infection. The primary factors influencing viral clearance or persistence are the potent and long-lasting immune responses facilitated by the activation of CD4+ and CD8+ cytotoxic T cells. By binding their T-cell receptors to human leukocyte antigen (HLA) molecules, which transfer the viral antigens' peptide fragments, both T cells are able to recognise the viral antigens expressed on the surface of infected hepatocytes [5]. A cluster of genes on chromosome 6p21 makes up the major histocompatibility complex region, which codes for the HLA glycoprotein. It is acknowledged that a significant determinant of vulnerability to autoimmune disorders is HLA [6].

The six main genes A, B, C, DR, DQ, and DP make up the HLA cluster. The first three genes in class I control how endogenous antigens are presented, but the remaining three genes in class II affect how exogenous antigens are presented, which in turn affects how different infectious diseases turn out. Due to the great polymorphism of HLA alleles between groups, there is variance in the immunological response [7]. **Shaker *et al.*** [8] showed a correlation between HLA alleles and the outcome of HCV infection. However, the correlation between the related alleles varies greatly throughout populations.

The purpose of this study was to determine whether HLA DPB1 expression and the severity of chronic hepatitis C infection in Egyptian patients are related.

PATIENTS AND METHODS

Study design and participants: This case-control study was conducted at the Gastroenterology and Hepatology Outpatient Clinic at the Internal Medicine Department in Helwan University Hospitals, Ain Shams University Hospitals and Tropical Medicine and Gastroenterology Outpatient Clinic, Qena Faculty of Medicine, South Valley University through the period from September 2019 to August 2020. Eighty-five adult participants were enrolled into this study. They were divided into three groups: **group 1 (**Cirrhotic

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HCV) included 20 cirrhotic patients with HCV, **group 2 (Chronic HCV)** included 20 chronic HCV patients and **group 3 (Control group)** included 45 healthy controls. Patients were diagnosed with chronic HCV if infection lasted for more than 6 months as determined by persistently positive detection of HCV RNA and antibodies in blood samples.

Exclusion criteria: Patients who had HCC, HBV infection and HIV infection or other chronic liver diseases (hemochromatosis, Wilson disease, alpha 1 antitrypsin deficiency and significant alcohol consumption).

Clinical and laboratory investigations: Every participant underwent a comprehensive clinical examination, a detailed history taking, and standard laboratory assessment. HLA DPB1 SNPrs3116996 polymorphism was assessed using Pure Link® Genomic DNA [Invitrogen, Life technologies (USA) and custom Taqman HLA DPB1 SNPrs3116996] genotyping assay (Applied Biosystems, USA) with a forward primer (5′>3′): GAAAGAGTGCCACACCTATTGC, reverse primer (5′>3′): AAATGTTGCTTGGCCTTTTG and Q probe (5′>3′): CTGAGCTAATAATTCATACAGTGAGAAAC.
HLA DPB1 SNPRs2071025 polymorphism was assessed using Pure Link® Genomic DNA (Invitrogen, Life technologies, USA) and custom Taqman HLA DPB1 SNPRs2071025 genotyping assay (Applied Biosystems, USA) with a forward primer (5′>3′): TTTCCTGGGTTCAAAGGTGAC, reverse primer (5′>3′): AAGGGAACACAGCACTGGAACT and Q probe (5′>3′): CCGTGTCCTACTGAGCCTCC. Automated allele calling was accomplished using Sequence Detection System (SDS) software (Applied Biosystems, USA). The results are presented as Multicomponent plot **(Fig 1).**

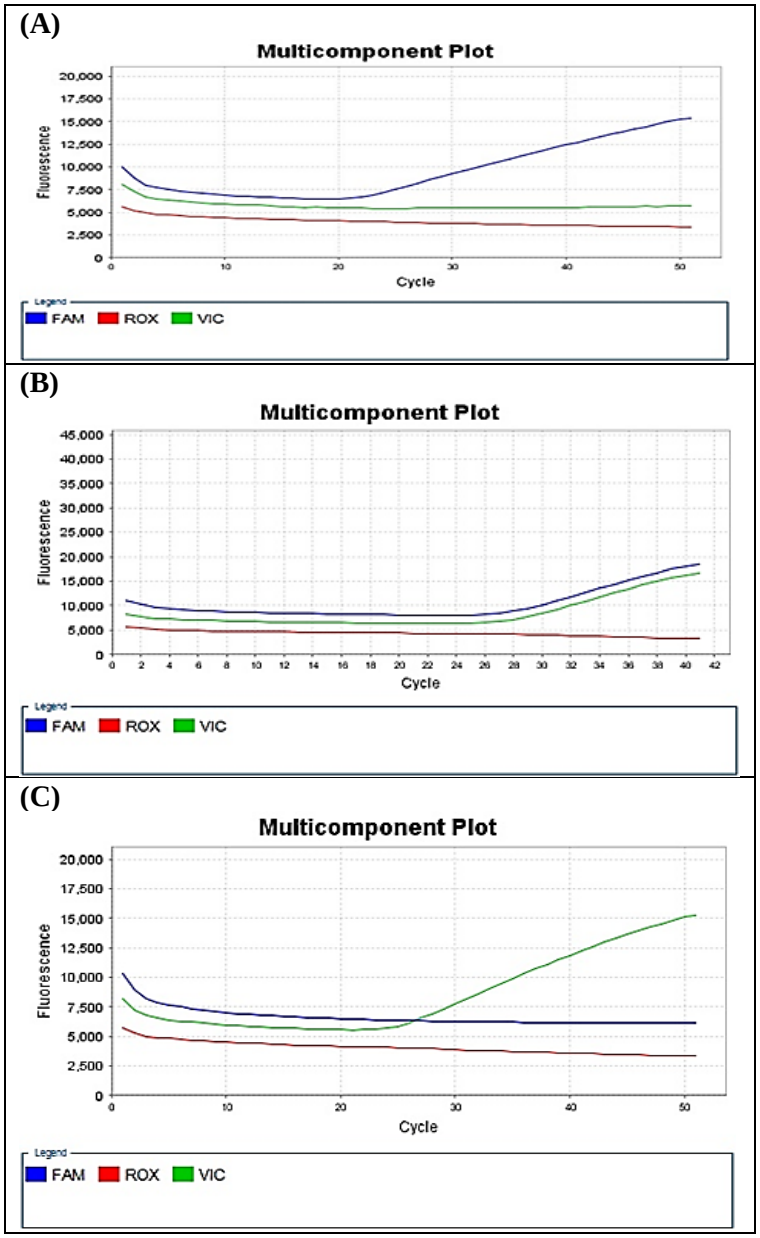

Fig. (1): Automated allele calling of HLA DPB1 (SNPrs3116996) & HLA DPB 1(SNPRs2071025) genes were performed using Sequence Detection System.

The results are presented as Multicomponent plot: **(A)** Homozygous A (VIC-dye fluorescence only meant homozygosity for allele 1(TT) (green). **(B)** 1st gen homozygous G (FAM-dye fluorescence only meant homozygosity for allele 2 (AA) (blue). **(C)** Gen heterozygous [Both VIC- and FAM-dye fluorescence meant allele1-allele2 heterozygosity (TA, AG) (both blue and green)].

Abdominal ultrasonography and transient elastography (fibro scan) were used in imaging investigations, and hepatologists with over five years of expertise measured liver stiffness. For each patient, the following serum liver fibrosis scores were determined using commonly available biological parameters: The upper limit of normal, or ULAN, is represented by the formula APRI (AST to platelets ratio index) = [(AST/ULN) x 100]/platelet count 10^9/L (AUC of 0.88 and 0.94 for severe fibrosis and cirrhosis, respectively) [9], and Fibrosis-4 (FIB-4) ={Plasma count (10^9/L) x √ALT (U/L) × Age (years) × AST Level (U/L)} (Fib-4 cut-off values of 1.45 or 3.25, respectively, to rule out or in severe fibrosis) [10].

The severity of liver disease was evaluated using the Model for End-Stage Liver Disease (MELD) [11], and Child-Turcotte-Pugh (CTP) [12].

Ethical approval: The Helsinki Declaration was followed in the course of this investigation. The Local Ethics Committees in Helwan and Qena Universities accepted the study protocol. Helwan local ethical committee code number: 24-2019 and Qena local ethical committee code number: SVU-MED-GIT023-4-23-2-549. Informed consents were obtained from all participants before participation in this work.

Statistical analysis

SPSS version 22 for Windows® was used to code, process, and analyse the collected data. The current investigation's data were presented as median and range, mean ± SD, and number and percent. One-way ANOVA (F), t test, Kruskal-Wallis test (KW) and Mann-Whitney (U) test were used to compare numerical data. Fisher's exact test and X^2 test were used to analyse categorical data, as needed. **P1:** the difference between cirrhotic HCV and chronic HCV groups. **P2:** the difference between chronic HCV and healthy control groups. **P3:** the difference between cirrhotic HCV and healthy control groups. P-values were considered statistically significant if they were less than.05.

RESULTS

The present study included 95 adults: cirrhotic HCV patients (n = 20), chronic HCV patients (n = 20), and healthy controls (n = 45). The mean ages of cirrhotic HCV patients, chronic HCV patients, and healthy volunteers were 53.35 ± 8.53, 45.65 ± 9.77, and 46.13 ±10.42 years respectively.

12 (60%) of cirrhotic HCV patients were males, 15 (75%) of chronic HCV patients were males, and 24 (53.33%) of healthy volunteers were males. With F/χ2 = 2.708 and a non-significant p. value (P = 0.258). Regarding demographic laboratory data of studied patient groups. Cirrhotic HCV patients showed decreased mean level of Hb, total WBC count and Plts. count compared to chronic HCV infected patients and healthy volunteer groups.

Concerning liver function tests, the median levels of ALT were 23.5 (13.3 – 85.5) in cirrhotic patients, 20.8 (12.2 – 37.4) in chronic HCV patients and 23.5 (11 – 32) in healthy controls; F/KW=1.447 with non-significant P. value (P= 0.485). The median levels of AST mean total bilirubin level and mean INR were higher in cirrhotic HCV patients, while mean serum albumin level was lower compared to chronic HCV patients and healthy control group.

Regarding the renal function tests, there was impaired renal function with raised serum creatinine level in cirrhotic HCV group compared to chronic HCV group and healthy control group. The mean level of total cholesterol of cirrhotic HCV patients, chronic HCV patients and healthy controls were 162.8 ± 20.8 mg/dl, 166.5 ± 16.37 mg/dl and 159.27 ± 20.48 mg/dl respectively with F= 0.969 and non- significant p. value (p= 0.384). The mean triglycerides levels of cirrhotic HCV patients, chronic HCV patients and healthy controls were 114.5 ± 21.75 mg/dl, 115.4 ± 20.12 mg/dl and 112 ± 21.4 (mg/dl), respectively with F= 0.214 and non- significant p. value (p= 0.808).

The mean random blood sugar (mg/dl) level in cirrhotic patients was 87.55 ± 15.63, 84.6 ± 8.42 in chronic HCV patients and 87.47 ± 9.16 in controls, F= 0.542, P= 0.583 (Table 1).

Table (1): Clinical and laboratory data in the studied groups

Variables	Cirrhotic HCV N=20	Chronic HCV N=20	Controls N=45	P1 value	P2 value	P3 value
Age (years)	53.35 ± 8.53	45.65 ± 9.77	46.13 ± 10.42	**0.016***	0.856	**0.008***
BMI (kg/m^2)	23.42 ± 1.03	24.22 ± 0.96	23.78 ± 0.98	**0.012***	0.099	0.179
Hemoglobin (g/dL)	11.54 ±1.41	13.01 ±0.96	13.53 ± 1.29	**0.003***	0.521	**<0.001****
WBCs (10^3/mL)	5.9 ± 1.36	7.61 ± 1.47	7.92 ± 1.78	**0.018***	0.681	**0.002***
Platelets (10^3/mL)	145.5 ± 34.26	254.95 ± 52.2	276.64 ± 54.41	**<0.001****	0.196	**<0.001****
Creatinine (mg/dl)	1.36 ± 0.17	1.07 ± 0.22	0.95 ± 0.18	**<0.001****	**0.016***	**<0.001****
Bilirubin (mg/dl)	2.2 ±0.54	0.72 ± 0.17	0.65 ± 0.14	**<0.001****	0.717	**<0.001****
Serum albumin (g/dL)	3.25 ±0.52	4.08 ± 0.33	4.12 ± 0.31	**<0.001****	0.62	**<0.001****
AST (U/L)	32.3 (20 – 215)	26.2 (13 – 39)	21.7 (13 – 28)	0.146	**0.004***	**<0.001****
INR	1.5 ± 0.36	1.09 ± 0.05	1.05 ± 0.08	**<0.001****	0.436	**<0.001****
PT (seconds)	16.48 ± 2.81	13.31 ± 0.38	12.89 ± 0.62	**<0.001****	0.284	**<0.001****
FIB-4	2.6 (0.95 – 7.07)	1.01 (0.45 – 1.68)	0.8 (0.33 – 0.98)	**<0.001****	**<0.001****	**<0.001****
APRI	0.56 (0.23 – 2.53)	0.24 (0.13 – 0.38)	0.2 (0.1 – 0.94)	**<0.001****	<0.999	**<0.001****

Data is represented as median and range: non parametric test.

P1: the difference between cirrhotic HCV and chronic HCV groups. **P2:** the difference between chronic HCV and healthy control groups. **P3:** the difference between cirrhotic HCV and healthy control groups.

Comparison between the studied groups regarding HLA-DPB1 (SNPrs3116996) genotypes and alleles revealed significantly higher frequency of HLA-DPB1 (SNPrs3116996) genotype TA in cirrhotic HCV patients in comparison with chronic HCV patients and healthy controls (40.0 % versus 15.0 % and 4.4 % respectively; p=0.002). In addition, cirrhotic HCV group had significantly higher frequency of A allele when compared with chronic HCV group and healthy controls (20.0 % versus 7.5 % and 2.2 % respectively; p<0.001). As regards the relation between genotype and development of cirrhosis among HCV patients, although statistically non-significant relation detected between genotype and development of cirrhosis among chronic HCV group, we found that TA genotype was more frequent in the cirrhotic HCV group (40%) than in the chronic HCV group (15%) with increased risk of cirrhosis among HCV patients by 3.78 folds. Also, statistically non- significant difference has been found between percentage of both A and T alleles in the cirrhotic HCV group and chronic HCV group. But, A allele was more frequent in cirrhotic HCV group (20%) than in the chronic HCV group (7.5%). A allele increased the risk of cirrhosis among HCV patients by 3.08 folds. By comparing between the chronic HCV patient group and the healthy controls, there was no significant difference regarding genotype and allele. But TA genotype was more frequent in the chronic HCV patients (15%) than in the controls (4.4%) and TA genotype increased risk of chronic HCV infection by 3.79 folds. A allele was more frequent in the chronic HCV patients (7.5%) than in the controls (2.2%), and A allele increased risk of chronic HCV infection by 3.57 folds. By comparing HCV cirrhotic patient group and control group. It was found that TA genotype was significantly more frequent in the HCV cirrhotic patients (40%) than in the controls (4.4%) (P value 0.007*) and TA increased risk of cirrhosis among HCV patients by 14.33 folds. A allele was more frequent in the HCV cirrhotic patients (20%) than in the control (2.2%) (P value 0.001**). Also, A allele significantly increased risk of cirrhosis among HCV patients by 11 folds (Table 2).

Table (2): Comparison between the studied groups regarding HLA DPB1 (SNPrs3116996) genotypes and allele:

HLA DPB 1 (SNPrs3116996) genotypes	Variables																	
	Study Groups						Study Groups				Study Groups				Study Groups			
	Cirrhotic HCV group N=20		Chronic HCV group N=20		Control group N=45		Cirrhotic HCV group N=20		Chronic HCV group N=20		Chronic HCV group N=20		Control group N=45		Cirrhotic HCV group N=20		Control group N=45	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
TA	8	40	3	15	2	4.4	8	40	3	15	3	15	2	4.4	8	40	2	4.4
TT	12	60	17	85	43	95.6	12	60	17	85	17	85	43	95.6	12	60	43	95.6
P-value (χ^2)	0.002* (13.513)						0.155				0.164				0.007*			
COR (95% CI)	-						3.78 (0.83 – 17.26)				3.79 (0.58 –24.75)				14.33 (2.68 – 76.63)			
A	8	20	3	7.5	2	2.2	8	20	3	7.5	3	7.5	2	2.2	8	20	2	2.2
T	32	80	37	92.5	88	97.8	32	80	37	92.5	37	92.5	88	97.8	32	80	88	97.8
P-value (χ^2)	<0.001**(11.782)						0.193				0.16				0.001**			
COR (95% CI)	-						3.08 (0.75 – 12.61)				3.57 (0.57 –22.24)				11 (2.2 – 54.56)			

COR Crude odds ratio, **CI** Confidence interval.

As shown in table (3), the comparison between the three study groups regarding HLA DPB1 (SNPRs2071025), genotypes and allele frequencies showed significantly higher frequencies of heterozygous mutant genotype AG in patients with cirrhotic HCV (45%) followed by chronic HCV group (25%) with the least frequencies in controls (8.9%).

The homozygous genotype GG is only detected in chronic HCV group. Regarding frequency of AA genotype, it was 70% in chronic HCV patients followed by cirrhotic HCV patients (55%) with the highest frequency of AA genotype (91.1%) in controls (p-value 0.001**).

In addition, there was significantly higher allele G frequency among the cirrhotic HCV patients (22.5%) followed by chronic HCV patients (17.5%) compared to controls (4.4%). Indicating that, G allele carry risk of cirrhosis development among HCV patients. By comparing between cirrhotic HCV patients and chronic HCV patients, it was found that AG genotype was more frequent in the cirrhotic HCV patients (45%) than in the chronic HCV patients (25%). AG genotype non-significantly increased risk of cirrhosis among HCV patients by 2.29 folds.

G allele was more frequent in the cirrhotic HCV patients (22.5%) than in the chronic HCV patients (17.5%). G allele increased risk of cirrhosis among HCV patients by 1.37 folds. Regarding genotype and allele frequencies in chronic HCV patients and controls, It was found that AG genotype was more frequent in the chronic HCV patients (25%) than in the control (8.9%) with increased risk of development of chronic HCV infection by 3.66 in healthy controls who got HCV infection. G allele was more frequent in the chronic HCV patients (17.5%) than in the controls (4.4%) indicating that G allele carry risk of chronic HCV development in healthy patients who got infected with HCV, with 4.56 folds.

There was statistically significant difference between the cirrhotic HCV patients and control group regarding genotype and allele. It was found that AG genotype was more frequent in the HCV cirrhotic patients (45%) than in the control (8.9%). AG genotype significantly increased risk of cirrhosis development post chronic HCV infection by 8.39 folds.

G allele was more frequent in the HCV cirrhotic patients (22.5%) compared to the controls (4.4%). G allele significantly increased risk of cirrhosis development among chronic HCV patients by 6.24 folds. In the cirrhotic HCV group, comparison between patients TA and TT genotypes regarding the laboratory data showed that the former group had significantly higher Child-Pugh score (10.38 ± 2.5 versus 6.75 ± 2.38; p=0.004), higher MELD score (18 (14.0 – 27.0) versus 10.5 (6.0 – 23.0); p=0.005), higher APRI score 1.11 (0.43 – 2.53 versus 0.46 (0.23 – 1.08); p=0.007 and higher FIB-4 score 4.52 (1.86 – 7.07) versus 2.17 (0.95 – 3.65); p=0.009.

Table (3): Comparison between the studied groups regarding HLA DPB1 (SNPRs2071025) genotypes and allele

HLA DPB1 (SNPRs2071025) genotypes	Variables																	
	Study Groups						Study Groups				Study Groups				Study Groups			
	Cirrhotic HCV group N=20		Chronic HCV group N=20		Control group N=45		Cirrhotic HCV group N=20		Chronic HCV group N=20		Chronic HCV group N=20		Control group N=45		Cirrhotic HCV group N=20		Control group N=45	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
AA	11	55	14	70	41	91.1	11	55	14	70	14	70	41	91.1	11	55	41	91.1
AG	9	45	5	25	4	8.9	9	45	5	25	5	25	4	8.9	9	45	4	8.9
GG	0	0	1	5	0	0	0	0	1	5	1	5	0	0	0	0	0	0
P-value (χ^2)	0.001** (14.479)						0.562				0.019				0.001**			
COR (95% CI)	-						2.29 (0.59 – 8.82)				3.66 (0.86 –15.58)				8.39 (2.17 – 32.44)			
A	31	77.5	33	82.5	86	95.6	31	77.5	33	82.5	33	82.5	86	95.6	31	77.5	86	95.6
G	9	22.5	7	17.5	4	4.4	9	22.5	7	17.5	7	17.5	4	4.4	9	22.5	4	4.4
P-value (χ^2)	0.002* (9.837)						0.781				0.034				0.001**			
COR (95% CI)	-						1.37 (0.45 – 4.12)				4.56 (1.25 –16.61)				6.24 (1.79 – 21.73)			

Concerning, AA and AG genotypes in cirrhotic HCV patient group, patients with AG genotype had marked reduction in the mean platelets count (144.0 ± 34.88), increase in the median total bilirubin level 2.1 (1.07 – 4) and significant lower mean albumin level 3.0 ± 0.51 compared to patients with AA genotype where mean platelets count were 146.73 ± 35.75, median total bilirubin level were 1.4 (0.42 – 6.3), and mean albumin level were 3.46 ± 0.45 with significant P = 0.047 (Table 4).

Table (4): Relation between HLA DPB1 (SNPrs3116996) and HLA DPB1 (SNPRs2071025) genotypes and the studied parameters among cirrhotic HCV patients

Variables (Mean±SD)	SNPrs3116996		p value	SNPRs2071025		p value
	TA	TT		AA	AG	
Age (years)	51.88 ± 9.95	54.33 ± 7.75	0.542	54.36 ± 7.51	52.11 ± 9.96	0.571
BMI (kg/m^2)	23.21 ± 1.19	23.56 ± 0.93	0.465	23.6 ± 0.77	23.2 ± 1.29	0.391
WBCs (10^3/mL)	6.13 ± 1.52	5.75 ± 1.41	0.683	5.24 ± 1.21	6.71 ± 1.62	0.091
Hemoglobin (g/dL)	11.04 ± 1.21	11.87 ± 1.48	0.206	11.59 ± 1.6	11.47 ± 1.23	0.851
Platelets (10^3/mL)	95.88 ± 23.75	178.58 ± 43.11	**0.011**	146.73 ± 35.75	144.0 ±34.88	0.944
Albumin (gm/dL)	2.94 ± 0.41	3.47 ± 0.5	**0.022**	3.46 ± 0.45	3.0 ± 0.51	**0.047***
Bilirubin (mg/dl)	2.7 (1.4 – 6.3)	1.15 (0.42 – 4.0)	**0.009**	1.4 (0.42 – 6.3)	2.1 (1.07 – 4)	0.382
ALT (U/L)	22.5 (18.0 – 85.5	24.0 (13.3 – 55.0)	0.643	24 (13.3 – 85.5)	21 (13.3 –55)	0.493
AST (U/L)	38.15 (24.0 –80.(	29.3 (20.0 –215.0)	0.113	35.6 (24 – 80.6)	30 (20 – 215)	0.113
Creatinine (mg/dl)	1.44 ± 0.11	1.31 ± 0.19	0.089	1.31 ± 0.17	1.42 ± 0.17	0.196
INR	1.84 ± 0.35	1.27 ± 0.24	**<0.001**	1.5± 0.36	1.49 ± 0.32	0.951
PT (seconds)	18.8 ± 2.64	14.93 ± 1.63	**0.001**	18.8 ± 2.64	16.37 ± 2.18	0.875
RBS (mg/dL)	90.13 ± 22.64	85.83 ± 9.35	0.562	90.13 ± 22.64	90.44 ± 8.35	0.469
Triglycerides (mg/dL)	116.38 ± 24.28	113.25 ± 20.92	0.762	116.38 ± 24.28	117.33 ± 22.18	0.612
Cholesterol (mg/dL)	165.63 ± 20.98	160.92 ± 21.38	0.633	165.63 ± 20.98	163.33 ± 14.96	0.917
Child-Pugh score	10.38 ± 2.5	6.75 ± 1.57	**0.004**	10.38 ± 2.5	9.11 ± 2.18	0.226
MELD	18.0 (14.0 – 27.0)	10.5 (6.0 – 23.0)	**0.005**	18.0 (14.0 – 27.0)	16 (10 – 23)	0.424
APRI	1.11 (0.43 –2.53)	0.46 (0.23 – 1.08)	**0.007**	1.11 (0.43 –2.53)	0.46 (0.33 –1.08)	0.254
FIB-4	4.52 (1.86 – 7.07)	2.17 (0.95 –3.65)	**0.009**	4.52 (1.86 –7.07)	2.28 (0.95 – 4.28)	0.271

Data is represented as median and range: non parametric test

Concerning, HLA DPB1SNPrs3116996 genotypes and alleles, 2 (3.8%) patients with TA genotype were F0, 3 (23.1%) were F1 and 8 (40%) were F4. While, in patients with TT genotype 50 (96.2%) were F0, 10 (76.9%) were F1 and 12 (60%) were F4; with most patients with TA genotypes had advanced fibrosis and most patients with TT genotype had no hepatic fibrosis, with significant P. value (P=0.001). Regarding, T allele, 2 (1.9%) patients were F0, 3 (23.1%) were F1 and 8 (25%) were F4. While, in patients with A allele; 102 (98.1%) were F0, 23 (76.9%) were F1 and 32 (75%) were F4 with significant P. value (P=0.001).

As regards, HLA DPB1SNPRs2071025 genotypes and alleles, AA, AG and GG genotypes, F0 were found in 46 (88.5%) of patients with AA genotype, 6 (11.5%) patients with AG genotype and none of patients with GG genotype. In addition, F1 were found in 9 (69.2%) of patients with AA genotype, 3 (23.1%) of patients with AG genotype and 1 (7.7%) of patients with GG genotype. And F4 were found in 11 (55%) of patients with AA genotype, 9 (45%) of patients with AG genotype and none of patients with GG genotype; with most patients with AG genotypes had advanced fibrosis, while most patients with AA genotypes had early or no fibrosis (P=0.001).

Regarding A allele, 98 (94.3%) patients were F0, 21(80.8%) were F1 and 31 (77.5%) were F4. While, in patients with G allele; 6 (5.7%) were F0, 5 (19.2%) were F1 and 9 (22.5%) were F4 with significant P. value (P=0.003) (Table 5).

Table (5): Relation between fibro scan results and HLA DPB1 (SNPrs3116996) and (SNPRs2071025) genotypes and alleles

SNPrs3116996	**F0 (N=52)**	**F1 (N=13)**	**F4 (N=20)**	χ^2	**P. value**
Genotypes: **TA** **TT**	 2 (3.8%) 50 (96.2%)	 3 (23.1%) 10 (76.9%)	 8 (40%) 12 (60%)	15.101	**<0.001***
Alleles: **T** **A**	 2(1.9%) 102(98.1%)	 3 (23.1%) 23(76.9%)	 8 (25%) 32 (75%)	13.393	**<0.001****
SNPRs2071025					
Genotypes: **AA** **AG** **GG**	 46 (88.5%) 6 (11.5%) 0 (0%)	 9 (69.2%) 3 (23.1%) 1 (7.7%)	 11 (55%) 9 (45%) 0 (0%)	MC	**<0.001****
Alleles: **A** **G**	 98 (94.3%) 6 (5.7%)	 21(80.8%) 5 (19.2%)	 31 (77.5%) 9 (22.5%)	8.854	**0.003***

MC Monte Carlo test, **χ2** Chi square for trend test

DISCUSSION

HLA-DPB is a member of HLA class II and is expressed by B lymphocytes, dendritic cells, and macrophages, among other antigen-presenting cells. As a result, it is essential to the immune system [13]. Clinical outcome after HCV infection differ greatly between individuals, about one quarter of patients have spontaneous resolution, whereas the remainder have persistent infection, which can lead to the development of liver cirrhosis and hepatocellular cancer [14-16]. Clinical outcome depends on many factors, as, viral genotypes, sex, age, BMI, race, and alcohol consumption [17-21]. Recently, HLA DPB1 expression is thought to play a significant influence in disease development in HCV-infected individuals [22].

In the current investigation, cirrhotic HCV patients had a considerably greater frequency of the HLA-DPB1 SNP rs3116996 genotype TA than chronic HCV patients and healthy controls. Furthermore, the cirrhotic HCV group exhibited a considerably greater frequency of the A allele than the chronic HCV group or healthy controls.

As far as we are aware, this is the second published work that relates HCV-related cirrhosis to **HLA-DPB1 SNP rs3116996** polymorphisms. Our findings are consistent with a Japanese research by **Hiramatsu *et al.* [22],** which showed that patients with HCV-related liver illness had higher levels of HLA-DPB1 gene expression and that these levels were connected with the course of the disease. For two SNPs (rs2071025 and rs3116996), the minor allele frequencies were significantly higher in individuals with liver cirrhosis and chronic liver disease due to HCV. It could be related to that, HCV infected patients with dangerous allele showed high level of antigen presentation by HLA-II DPB1 molecules with subsequent potent antigen-specific CD4+ T-helper cell responses [23].

In this study, HLA DPB 1 (SNPrs3116996) **T** allele was detected in 97.8% of healthy subjects. So, it may have a protective role against development of chronic HCV infection and deterioration of post-HCV liver cirrhosis. This comes in agreement with **Koukoulioti *et al.* [24]** who found in their study that **T** allele may have a protective role.

In this study, highest frequency of heterozygous mutant genotype **AG** was detected in patients with cirrhotic HCV followed by chronic HCV group with the least frequency in controls**.** The homozygous genotype **GG** is only detected in chronic HCV group, but the homozygous **AA** genotype showed high frequency in controls and least frequency in cirrhotic patients. In addition, the highest frequency of allele **G** was found in the cirrhotic HCV patients with the least frequency in the controls. So, individuals with **AG** and **AA** genotypes have a high possibility of persistent viral infection and rapid deterioration of the liver condition. On the other hand, patients with **AA** genotype have a high possibility of viral clearance and low risk of liver cirrhosis deterioration. Also, **G** allele carries risk of cirrhosis development among HCV patients. This comes in agreement with **Hu *et al.* [25]** study and considered that either persistent infection and deterioration of liver condition or spontaneous viral clearance and slow risk of deterioration of liver condition, is mainly related to different **T** helper cells and cytotoxic **T** cell response with different genotype predominance.

In this study, regarding **HLA DPB1 (SNPrs3116996) gene polymorphism,** cirrhotic patients with **TA** genotype showed marked impairment in different laboratory parameters, Child-Pugh score, MELD score, APRI score and FIB-4 score compared to the cirrhotic patient with **TT** genotype. The association between HLA-DPB1 polymorphisms and deteriorated clinical performance and extra-hepatic manifestations of HCV was previously reported. In the study of **Naruse and Inoko [26]**, the authors identified an association between HLA-DRB1*0901-DQB1*0303 haplotype and hypertrophic post HCV cardiomyopathy. In a Chinese research, the association between the HLA-DPA1 (rs3077) and HLA-DPB1 (rs9277534) polymorphisms with the risk of developing anti-F antibodies or chronic HCV was investigated. The findings showed that rs3077 considerably raised the chance of developing a chronic HCV infection. Additionally, rs3077 helped to lower the chance of developing anti-F antibodies [27].

In our study, Concerning **HLA DPB1 (SNPRs2071025)** gene polymorphism in cirrhotic patients, we found that patients with **AG** genotype had marked reduction in the mean platelets count, mean albumin level and increase in the median total bilirubin level compared with patients with **AA** genotype. Such findings are in agreement with **Ghazy *et al.* [23]** study on Egyptian patients with HCV infection. In their study they found that AG variants were higher among HCV infected patients and this variant may be regarded as a hereditary risk factor for HCV infection and disease progression with failure to response to DAA therapy.

Study limitation:

The current research's primary limitation was its limited sample size (doing a study with a large number of patients is quite expensive). Further research with a larger patient population is required to validate our findings.

CONCLUSION

This study carried an evidence of association between **HLADPB1 SNP rs3116996** genotype **TA** and **HLADPB1 SNP rs2071025** genotype **AG** and a risk of liver cirrhosis development in patients with chronic HCV infection and deterioration of clinical and biochemical parameters.

Contributions:

Study design (Elsayed Ibrahim Abdelrhman Ali, Samia A. Abdo Gamie, Enas M. Foda, Samah Ahmed

Bastawy, Heba Ahmed Osman, Abeer Mohamed Mahmoud Sabry), **data management and analysis** (Elsayed Ibrahim Abdelrhman Ali, Samia A. Abdo Gamie, Enas M. Foda, Samah Ahmed Bastawy, Heba Ahmed Osman, Abeer Mohamed Mahmoud Sabry), **data collection** (Elsayed Ibrahim Abdelrhman Ali, Samia A. Abdo Gamie, Enas M. Foda, Samah Ahmed Bastawy, Heba Ahmed Osman, Abeer Mohamed Mahmoud Sabry), **data interpretation** (Elsayed Ibrahim Abdelrhman Ali, Samia Ali Abdo Gamie, Enas M. Foda, Samah Ahmed Bastawy, Heba Ahmed Osman, Abeer Mohamed Mahmoud Sabry), **drafting the manuscript** (Elsayed Ibrahim Abdelrhman Ali, Samia A. Abdo Gamie, Enas M. Foda, Samah Ahmed Bastawy, Heba Ahmed Osman, Abeer Mohamed Mahmoud Sabry), **critical revision for essential intellectual substance** (Elsayed Ibrahim Abdelrhman Ali, Samia A. Abdo Gamie, Enas M. Foda, Samah Ahmed Bastawy, Heba Ahmed Osman, Abeer Mohamed Mahmoud Sabry) and **study supervision** (Elsayed Ibrahim Abdelrhman Ali, Samia A. Abdo Gamie, Enas M. Foda, Samah Ahmed Bastawy, Heba Ahmed Osman, Abeer Mohamed Mahmoud Sabry). The submitted article draft has been approved by all authors.

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