Research Article
Open Access
CrossMark

Single nucleotide polymorphism of the promotor region of Interleukin-10 gene did not show significant association with spontaneous clearance of hepatitis C virus infection in Egyptian patients

Abstract

Introduction: Accurate etiologies of spontaneous clearance of HCV infection are hard to be clarified. However, it is proposed that genetically determined interleukin-10 (IL-10) changes play an important function in HCV elimination. Individuals who are homozygous for IL-10 gene promoter region 1082 (G/A; rs1800896) genotype AA have significantly lower levels of plasma IL-10 levels with better capability of spontaneous viral clearance (SVC); while those with GG genotype have two folds greater level of IL-10 than GA or AA persons, leading to diminished capability of SVC.

Aims: The study aimed at identifying the IL-10 gene promoter region 1082 (G/A; rs1800896) polymorphism as a possible predictor of SVC of HCV infection in Egyptian patients and its relation with the viral load and the degree of liver injury in chronic hepatitis C (CHC) patients.

Methods: The study was conducted on two groups; group I which included 100 cases of CHC defined as detectable anti-HCV antibodies and detectable serum HCV RNA for ≥ 6 months and group II which included 50 cases of SVC defined as detectable anti-HCV antibodies for ≥ 6 months and undetectable HCV RNA in two consecutive tests, 3 months apart, without previous antiviral therapies. HCV antibody testing was done using 4th generation enzyme-linked immuno sorbent assay (ELISA) technique. HCV-RNA quantification was done using polymerase chain reaction (PCR) technique. Detection of IL10 1082 (G/A; rs1800896) polymorphism was done by PCR restriction fragment length polymorphism (RFLP) technique. Complete blood count (CBC); serum albumin, serum bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), international normalized ratio (INR), serum creatinine and HBsAg, were done for all participants. Child-Turcotte-Pugh (CTP) class & FIB4 score were calculated. Abdominal ultra-sonography (US) and contrast enhanced computed tomography (CT) of the abdomen were done for all participants.

Results: In group I, the age was 53.6±11.2 years old, males represented 51% and females represented 49%. In group II, the age was 52.8±8.6 years old, males represented 48% and females represented 52%. No significant differences were found regarding age and gender (p=0.658, 0.729 respectively). Group I showed significantly higher frequency of hepatomegaly, splenomegaly & liver cirrhosis ($p < 0.001$) and significantly lower platelet count (PLC) (183.5±61.1 versus 220.3±67.2 ×109/L, p=0.006). Group I showed significantly higher ALT, AST & FIB4 score (p <0.001, 0.002, <0.001 respectively). Group I had median viral load of 555640 IU/mL. Applying Hardy Weinberg (HW) equation, IL10 1082 (G/A; rs1800896) genotypes in both groups were in HW equilibrium (HW p=0.845&0.861 respectively). In Group I, genotype AA represented 72%, AG represented 26% & GG represented 2%, while in Group II, genotype AA represented 62%, AG represented 34% & GG represented 4% ,with insignificant differences between both groups (p=0.272, 0.415 & 0.216 respectively). In Group I, no significant differences regarding HCV RNA load ,CTP class, PLC , FIB4 score ,were found between the three genotypes (p=0.191, 0.086, 0.754,0.829, 0.187 respectively). Multivariate logistic regression analysis showed that IL10 1082 (G/A; rs1800896) genotypes was not a significant predictor of SVC of HCV infection in Egyptian patients (p=0.216.OR=1.322, 95% CI=0.849-2.058).

Conclusion: Single nucleotide polymorphism of the promoter region of the IL-10 gene, 1082 (G/A; rs1800896), did not show significant association with the spontaneous clearance of hepatitis C virus infection in Egyptian patients. In chronic hepatitis C patients, this SNP had no significant association with the serum HCV viral load, CTP class, PLC or FIB4 score.

Keywords: chronic hepatitis C, single nucleotide polymorphism, IL-10, spontaneous viral clearance, the promoter region, 1082 G/A; rs1800896; Egypt

Volume 14 Issue 6 - 2023

Fatma Waly,[1] Maysaa El Sayed Zaki,[1] Hany Shabana,[2] Zakaria Lotfy[1]
[1]Clinical Pathology Dep., Faculty of Medicine, Mansoura University, Mansoura, Egypt
[2]Internal Medicine Dep., Hepatology and Gastroenterology Unit, Specialized Medical Hospital, Faculty of Medicine, Mansoura University, Mansoura, Egypt

Correspondence: Hany Shabana, Assistant Professor of Internal Medicine, Hepatology and Gastroenterology Unit, Specialized Medical Hospital, Faculty of Medicine, Mansoura University, Mansoura, Egypt, Tel +201060396985, Email hra2010@live.com

Received: November 23, 2023 | **Published:** December 14, 2023

Abbreviations: ANOVA, analysis of variance AHC, acute hepatitis C; ALT, alanine aminotransferase; AFP, Alpha fetoprotein; AFP-L3, lens culinaris agglutination reactive AFP; AFU, alpha-l-fucosidase; AIH, autoimmune hepatitis; AST, aspartate aminotransferase; AUC, area under curve; CBC, complete blood count; CD, cluster differentiation; CI, confidence interval; CHC, chronic hepatitis C; CRP, C-reactive protein; CT, computed tomography; CTP, Child -Turcotte -Pugh; CSIF, cytokine synthesis inhibitory factor; DAAs, Direct acting antiviral agents; DM, Diabetes Mellitus; EDHS, Egyptian demographic health survey; ELISA, Enzyme-Linked immuno Sorbent Assay; HBV, Hepatitis B Virus; HCC, Hepatocellular Carcinoma; HCV, Hepatitis C Virus; HW, Hardy Weinberg; HIV, human immunodeficiency virus; IFN-γ, Interferon gamma; INR, International Normalized Ratio; IL-10, Interleukin-10; MDCT, Multi Detector CT; NASH, Non-Alcoholic Steatohepatitis; N/L, neutrophil/lymphocyte; NPV, negative predictive value; OR, Odds Ratio; OS, Overall Survival; PCR, polymerase chain reaction; PLC, platelet count; PT, prothrombin time; PPV, positive predictive value; RBCs, red blood cells; RFLP, restriction fragment length polymorphism; ROC, Receiver operating characteristic curve; SD, standard deviation; SE, standard error; SMH, specialized medical hospital; Sn, sensitivity; SNPs, single-nucleotide polymorphisms; Sp, Specificity; SVC, spontaneous viral clearance; TPO, thrombopoietin; TNF-α, tumor necrosis factor alpha; Th2, T-helper 2; US, ultra sound; VL, viral load; WBCs, white blood cells

Introduction

Hepatitis C virus (HCV) infection in Egypt had the greatest frequency worldwide (14.7% of the population). Its incidence in Egypt ranged from 0.8 to 6.8 per 1,000 person-years. The predominant genotype in Egypt is type four (73% of the cases). The source, development and dynamics are hard to determine.[1] Risk factors for HCV infection involve history of anti-schistosomal injection therapy prior to 1986, old age, male sex and rural residency.[2] Chronic HCV infection usually causes chronic inflammation with subsequent hepatic damage and fibrosis which progresses to cirrhosis and finally hepatocellular carcinoma (HCC).[3] However, a small number of cases (20%) mount an immune response that efficiently overcomes the infection.[4] Patients with acute HCV infection who undergo spontaneous viral clearance (SVC) had positive anti HCV antibody for more than 6 months with undetectable HCV RNA in the serum; without therapy. Since oscillations in HCV RNA detection are commonly noticed in the initial stage of HCV infection, two successive negative HCV RNA test results were needed to confirm SVC.[5] Cytokines influence the natural course of HCV infection. They represent a large family of molecules, that have an essential role in the beginning and controlling of immune response, and consequently, cytokines might influence HCV infection outcomes.[6] Interleukin 10 (IL-10) is a multifunctional cytokine that down regulates the synthesis of pro-inflammatory cytokines and has a modulatory effect on hepatic fibrogenesis. IL-10, also known as human cytokine synthesis inhibitory factor (CSIF), is an anti-inflammatory cytokine. In humans, IL-10 is encoded by the IL10 gene which is located on chromosome 1 and comprises 5 exons. The IL-10 protein is a homodimer; each of its subunits is 178-amino-acid long.[7] IL 10 is mainly created by monocytes with little amounts by lymphocytes, type 2 T helper cells (Th2), mast cells, CD4+CD25+Foxp3+ regulatory T cells, and in some subset of stimulated T cells and B cells.[8] Persons with IL-10 1082 (G/A; rs1800896) GG genotype create greater values of IL-10 than GA or AA persons. Moreover, IL-10 is a potent suppressor of pro-inflammatory mediators including IL-1, TNF-α, IL-6 and IFN-γ. It was thought that, higher creation of IL-10 might enhance viral escape by down regulation of the protective Th1 response.[9]

Aim of work

The study aimed at identifying the IL-10 gene promoter region 1082 (G/A; rs1800896) polymorphism as a possible predictor of spontaneous viral clearance of HCV infection and its relation with the viral load and the degree of liver injury in Egyptian patients with chronic hepatitis C.

Type, place & duration of the study

This study was a single center, case- control study. The work was conducted on two groups; group I which included 100 cases of chronic hepatitis C infection (CHC) defined as detectable anti-HCV antibodies and detectable serum HCV RNA for ≥ 6 months and group II which included 50 cases of spontaneous viral clearance (SVC) defined as detectable anti-HCV antibodies for ≥ 6 months, and undetectable HCV RNA in two consecutive tests, 3 months apart, without previous antiviral therapies. All participants were recruited from outpatient clinic of Hepatology & Gastroenterology unit, Internal Medicine department, Specialized Medical Hospital, Faculty of Medicine, Mansoura University. The molecular study was carried out in molecular biology laboratory of Clinical Immunology unit, Clinical Pathology Department, Mansoura University, Mansoura, Egypt. The study extended from 1/2017 to 1/2018.

Ethical considerations

Study protocol was accepted by the medical ethics research committee, Faculty of Medicine, Mansoura University (Code number: MS/16.11.04). Each participant in the study, after assuring confidentiality, has given an informed written consent.

Inclusion criteria

The enrolled participants were adult patients with chronic hepatitis C defined as detectable anti-HCV antibodies and serum HCV RNA for more than 6 months of follow up and adult patients with detectable anti-HCV antibodies, who underwent spontaneous viral clearance, with undetectable HCV RNA in two consecutive tests over a minimum time interval of 3 months, within 12 months of follow up without treatment.

Exclusion criteria

The exclusion criteria were HBV or HIV co-infection, Alcohol ingestion, other etiologies of chronic liver disease, patients with HCV related HCC and patients with other malignancies (Figure 1).

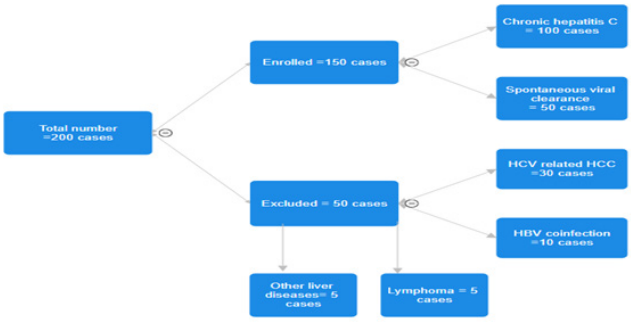

Figure 1 Study flowchart.

Methods

All enrolled participants were subjected to thorough clinical history, full clinical examination and laboratory investigations. CBC including total leucocyte count (TLC), neutrophil / lymphocyte N/L) ratio and platelet count (PLC) was done by automated blood cell counter (Sysmex XS-500i). Serum albumin, total bilirubin, alanine aminotransferase (ALT); aspartate aminotransferase (AST) and creatinine were done by clinical chemistry analyzer (Cobas C 311). INR was done using Behnk Elektronik Coagulator. Anti HCV antibodies, Anti HIV antibodies and HBs-Ag were done by 4th generation ELISA technique. Serum level of HCV-RNA was measured by polymerase chain reaction (PCR) using Applied Biosystems Step One Real-Time PCR System, Thermal Cycling Block. Evaluation of the degree of liver fibrosis and cirrhosis was done by FIB4 score which was calculated using the following formula: (Age × AST) / (Platelets × sqr ALT)). Child -Turcotte-Pugh (CTP) score was calculated. CTP class was considered class A if the score was 5-6 points, class B if score was 7-9 points and class C if the score was 10-15 points (Table 1).

Table 1 CTP score & class of the severity of liver disease

Parameter	1 point	2 points	3 points
Albumin (mg/dl)	>3.5	2.8-3.5	< 2.8
Bilirubin (mg/dl)	<2	3-Feb	>3
INR	<1.7	1.7-2.3	>2.3
Ascites	No	Mild	Moderate, severe
Hepatic encephalopathy	none	Grade 1-2	Grade 3-4

Molecular study

All enrolled participants were subjected to molecular study regarding IL-10 gene promoter region 1082 (G/A; rs1800896) polymorphism. DNA was isolated from whole blood by Gene JET Whole Blood Genomic DNA Purification Mini Kits from Thermo

Citation: Waly F, El-Sayed Zaki M, Shabana H, et al. Single nucleotide polymorphism of the promotor region of Interleukin-10 gene did not show significant association with spontaneous clearance of hepatitis C virus infection in Egyptian patients. *Gastroenterol Hepatol Open Access.* 2023;14(6):181–189.
DOI: 10.15406/ghoa.2023.14.00565

Scientific (lot 00132789).Ten ml of venous blood samples were collected from peripheral vein by clean vein puncture. Samples were stored at (-20◦c) until the time of molecular biology techniques for detection of IL10 (1082) A/G polymorphism by PCR restriction fragment length polymorphism (RFLP) technique.

Statistical analysis

Data analysis was performed by SPSS software, version 25 SPSS Inc., PASW statistics for windows version 25 (SPSS Inc., PASW statistics for windows version 25. Chicago: SPSS Inc.). Qualitative data were described using number and percent. Quantitative data were described using median for non-normally distributed data and mean ± Standard deviation (SD) for normally distributed data, after testing normality using Kolmogrov-Smirnov test. Chi-Square, Fisher exact test, Monte Carlo tests were used to compare qualitative data as appropriate. Kruskal Wallis was used to compare between more than 2 studied groups, respectively for non-normally distributed data. One Way ANOVA test was used to compare more than 2 independent groups with Post Hoc Tukey test to detect pair-wise comparison. Logistic regression analysis was used for detection of SVC predictors using generalized linear models. Deviations from Hardy–Weinberg equilibrium expectations were determined using the chi-squared test. Significance of the obtained results was judged at the ≤0.05 value.

Results

The mean age of CHC group was 53.6 years, they were 51 males (51%) and 49 females (49%). The mean age of SVC group was 52.8 years, they were 24 males (48%) and 26 females (52%). There were insignificant differences regarding age, gender, anthropometric data, DM and smoking between both groups. CHC patients showed significantly higher frequency of hepatomegaly, splenomegaly and liver cirrhosis. Patients with CHC had CTP class A in 92% & CTP class B in 7%. All SVC subjects had CTP class A (Table 2).

Table 2 Comparison of baseline clinical & radiological data of the studied groups

			Chronic hepatitis C N=100		Spontaneous viral clearance N=50		*P*
Age (years)		Mean ±SD	53.6	11.2	52.8	8.6	0.658
Males		N, %	51	51	24	48	0.729
Females		N, %	49	49	26	52	
Weight (kg)		Mean ±SD	88.1	11.1	88.2	13.4	0.985
Height (cm)		Mean ±SD	166.5	6.9	167.2	7.7	0.561
BMI (Kg/m^2)		Mean data ±SD	31.8	4.1	31.5	4.2	0.611
Smoking		N, %	33	33	13	26	0.381
DM		N, %	28	28	7	14	0.056
Hepatomegaly		N, %	22	22	0	0	<0.001
Splenomegaly		N, %	32	32	0	0	<0.001
Cirrhosis		N, %	35	35	0	0	<0.001
Ascites		N, %	6	6	0	0	0.178
CTP class	A	N, %	92	92	50	100	0.096
	B	N, %	7	7	0	0	

Table 3 Comparison of laboratory data of the studied groups

		Chronic hepatitis C N=100		Spontaneous viral clearance N=50		*P*
TLC (10^9/L)	Mean ±SD	7.4	2.3	5.6	1.5	0.129
Neutrophil (%)	Mean ±SD	50.5	11.4	47.6	11.3	0.142
Lymphocytes (%)	Mean ±SD	39.1	11.3	40.7	9.9	0.389
N/L ratio	Mean ±SD	1.5	0.5	1.5	0.3	0.781
Hemoglobin (g/dl)	Mean ±SD	13.1	2.2	12.5	1.5	0.068
Platelets (×10^9/L)	Mean ±SD	183.5	61.1	220.3	67.2	0.006
INR	Mean ±SD	1.1	0.1	1.1	0.1	0.341
Glucose (mg/dl)	Mean ±SD	119.2	53.5	119.5	37.8	0.14
AST (U/L)	Mean ±SD	49.5	36.1	27.3	13	<0.001
ALT (U/L)	Mean ±SD	47.4	34	31	14.2	0.002
FIB4 score	Mean ±SD	2.9	3	1.3	0.5	<0.001
Albumin (mg/dl)	Mean ±SD	4.2	0.6	4.3	0.4	0.214
Bilirubin (mg/dl)	Mean ±SD	0.8	0.2	0.7	0.3	0.156
Creatinine (mg/dl)	Mean ±SD	0.9	0.3	0.9	0.2	0.327
VL (IU/mL)	Median, range	555640	6500-800114000	-	-	-
Low VL	N, %	17	17%	-	-	-
Moderate VL	N, %	36	36%	-	-	-
High VL	N, %	47	47%	-	-	-

Citation: Waly F, El-Sayed Zaki M, Shabana H, et al. Single nucleotide polymorphism of the promotor region of Interleukin-10 gene did not show significant association with spontaneous clearance of hepatitis C virus infection in Egyptian patients. *Gastroenterol Hepatol Open Access*. 2023;14(6):181–189. DOI: 10.15406/ghoa.2023.14.00565

CHC patients had significantly higher ALT, AST & FIB4 score. They also had significantly lower PLC than SVC subjects. Otherwise, no significant differences were found regarding laboratory data of CHC and SVC groups. Median viral load (VL) in CHC group was 555640 IU/mL, ranging from 6500 to 800114000 IU/mL; with 17% of the cases had low viral load, 36% had moderate viral load and 47% had high viral load (Figure 2).

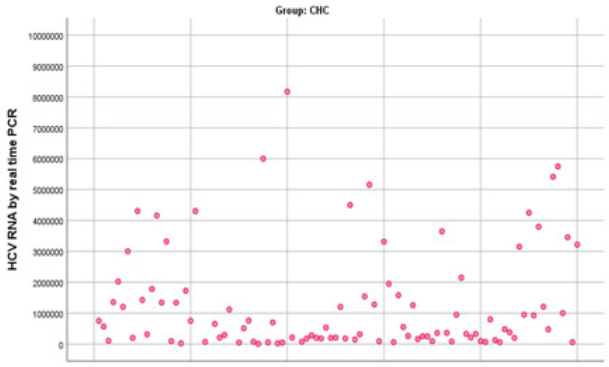

Figure 2 VL in CHC group.

Deviations from Hardy–Weinberg equilibrium HWE) expectations were determined using the chi-squared test. This sample of individuals was randomly selected from population in Dakahlia Governorate, Egypt. Applying Hardy Weinberg equation revealed that rs1800896 genotypes in CHC as well as in SVC groups were in HWE (Table 4).

Table 4 Assessment of Hardy Weinberg equilibrium in the studied groups

	Chronic hepatitis C N=100		Spontaneous viral clearance N=50	
	Observed	Expected	Observed	Expected
AA	72	72.3	31	31.2
AG	26	25.5	17	16.6
GG	2	2.3	2	2.2
HW *p*	0.845		0.861	

IL10 rs1800896 AA genotype (ancestral or major genotype) showed frequency of 72% in CHC group and 62% in SVC group. AG genotype showed frequency of 26% in CHC group and 34% in SVC group. The alternative genotype (GG) showed frequency of 2% in CHC group and 4% in SVC group. Dominant model (AG+GG) showed frequency of 28% in CHC group and 38% in SVC group. Calculation of allele frequency in studied groups revealed that the ancestral or major allele (A) showed frequency of 85% in CHC group and 79% in SVC group, while alternative or minor allele (G) showed frequency of 15% in CHC group and 21% in SVC group. Taking AA as the reference genotype and A as the reference allele; IL10 (rs1800896) genotypes and alleles did not show significant association with SVC (p=0.272, 0.415, 0.216, 0.192 respectively) (Table 5 & Figure 3).

Table 5 Comparison of rs1800896 genotypes and alleles of the studied groups

	CHC N=100		SVC N=50		p	OR	95% CI
AA	72	72	31	62	-	1	(Reference)
AG	26	26	17	34	0.272	1.292	0.818-2.040
GG	2	2	2	4	0.415	1.685	0.481-5.906
AG+GG	28	28	19	38	0.216	1.322	0.849-2.058
A	170	85	79	79	-	1	(Reference)
G	30	15	21	21	0.192	1.506	0.812-2.795

Table 6 shows that there were insignificant differences regarding clinical and radiologic data of IL10 (-1082 A/G) genotypes and alleles in CHC group.

Table 7 shows that there was insignificant differences regarding the laboratory data between IL10 (-1082 A/G) genotypes and alleles in CHC group.

Table 8 shows that there was insignificant differences regarding clinical and radiologic data of IL10 (-1082 A/G) genotypes and alleles in SVC group.

Table 9 shows that there was insignificant differences regarding laboratory data of IL10 (-1082 A/G) genotypes and alleles in SVC group.

Logistic regression analysis was conducted for prediction of SVC using gender, BMI, N/L ratio, INR, AST, ALT, FIB4, IL10 genotypes and alleles as covariates. Lower ALT, AST and FIB4 were significant predictors of SVC in univariable analysis. However, taking significant covariates into multivariate analysis revealed that only lower FIB4 was considered significant predictor of SVC (Table 10). Logistic regression analysis was conducted for prediction of CHC using gender, BMI, N/L ratio, INR, AST, ALT, FIB4, IL10 genotypes and alleles as covariates. Higher ALT, AST and FIB4 were significant predictors of CHC in univariable analysis. However, taking significant covariates into multivariate analysis revealed that only higher FIB4 was considered significant predictor of CHC.[10]

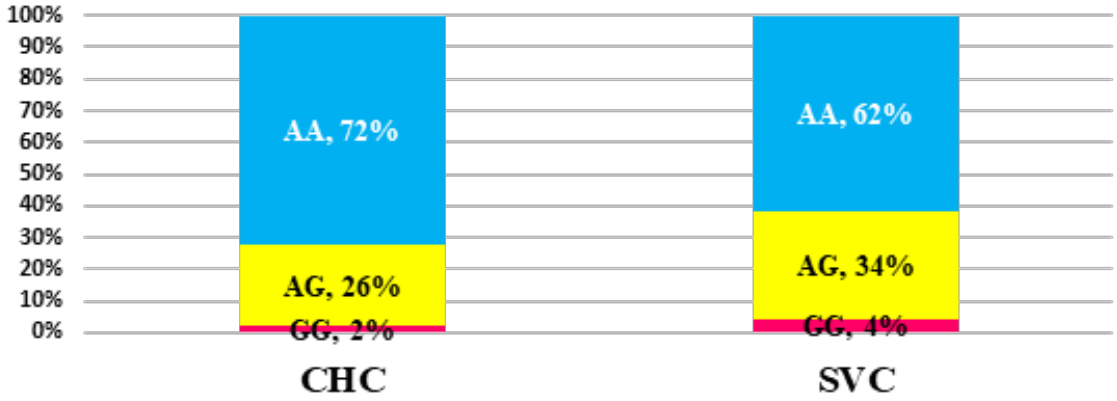

Figure 3 IL10 rs1800896 genotypes in CHC and SVC groups.

Citation: Waly F, El-Sayed Zaki M, Shabana H, et al. Single nucleotide polymorphism of the promotor region of Interleukin-10 gene did not show significant association with spontaneous clearance of hepatitis C virus infection in Egyptian patients. *Gastroenterol Hepatol Open Access*. 2023;14(6):181–189. DOI: 10.15406/ghoa.2023.14.00565

Table 6 Comparison of clinical and radiologic data of IL10 (-1082 A/G) genotypes and alleles in CHC group

CHC		AA N=72	AG N=26	GG N=2	AG+GG N=28	A N=170	G N=30	*P1*	*P2*	*P3*
Age (years)	Mean ±SD	52.6 11.4	55.8 10.2	60.5 10.6	56.1 10.1	53.1 11.3	56.4 10.1	0.305	0.151	0.127
Males	N %	38 52.8	12 46.2	1 50	13 46.4	88 51.8	14 46.7	0.834	0.568	0.607
Females	N %	34 47.2	14 53.8	1 50	15 53.6	82 48.2	16 53.3			
Weight (kg)	Mean ±SD	88.1 11.4	88.5 10.7	85 7.1	88.3 10.4	88.2 11.3	88.1 10.2	0.909	0.935	0.969
Height (cm)	Mean ±SD	166.1 6.8	167.5 7.6	166.5 3.5	167.5 7.4	166.4 6.9	167.4 7.2	0.677	0.39	0.443
BMI(Kg/m^2)	Mean ±SD	31.9 4.3	31.5 3.7	30.7 3.8	4.3 3.6	31.9 4.2	31.5 3.6	0.852	0.619	0.582
Smoking	N %	24 33.3	8 30.8	1 50	9 32.1	56 32.9	10 33.3	0.851	0.909	0.966
DM	N %	20 27.8	8 30.8	0 0	8 28.6	48 28.2	8 26.7	0.898	0.937	0.86
Hepatomegaly	N %	17 23.6	5 19.2	0 0	5 17.9	39 22.9	5 16.7	0.872	0.533	0.444
Splenomegaly	N %	22 30.6	10 38.5	0 0	10 35.7	54 31.8	10 33.3	0.528	0.62	0.865
Cirrhosis	N %	25 34.7	9 34.6	1 50	10 35.7	59 34.7	11 36.7	0.904	0.926	0.836
Ascites	N %	2 2.8	4 15.4	0 0	4 14.3	8 4.7	4 13.3	0.073	0.051	0.086
CTP class A	N %	69 95.8	21 80.8	2 100	23 82.1	159 93.5	25 83.3	0.191	0.086	0.13
CTP class B	N %	3 4.2	4 15.4	0 0	4 14.3	10 5.9	4 13.3			

P1= comparison between AA, AG GG; P2= comparison between AG + GG versus AA; P3= comparison between alleles.

Table 7 Comparison of laboratory data of IL10 (-1082 A/G) genotypes and alleles in CHC group

CHC		AA N=72	AG N=26	GG N=2	AG+GG N=28	A N=170	G N=30	P1	P2	P3
TLC (×109/L)	Mean ±SD	7.5 1.1	6.9 2.1	7.8 1.2	6.9 2.1	7.5 5.7	7 2.5	0.862	0.624	0.686
Neutrophil (%)	Mean ±SD	51.5 11.5	47.5 10.8	52 12.7	47.8 10.8	50.9 11.5	48.1 10.8	0.306	0.148	0.217
Lymphocytes (%)	Mean ±SD	38.6 11.8	40 10.1	42.5 4.9	40.1 9.8	38.8 11.6	40.3 9.6	0.791	0.537	0.501
N/L ratio	Mean ±SD	1.6 0.5	1.2 0.4	1.2 0.4	1.3 0.3	1.6 1.2	1.3 0.6	0.375	0.16	0.174
Hemoglobin (g/dl)	Mean ±SD	12.9 2.3	13.2 1.9	15.5 0.7	13.4 1.9	13 2.3	13.6 2	0.266	0.373	0.222
Platelets (×109/L)	Mean ±SD	180.4 51.6	193.3 63.5	167 16.9	191.5 60.7	182.4 81.6	189.9 78.2	0.754	0.542	0.642
INR	Mean ±SD	1.1 0.1	1.1 0.1	1.1 0.1	1.1 0.1	1.1 0.1	1.1 0.1	0.868	0.807	0.916
Glucose (mg/dl)	Mean ±SD	121.7 58.8	114.8 37.4	86.5 0.7	112.8 36.7	120.6 55.8	111 36.1	0.189	0.689	0.364
AST (U/L)	Mean ±SD	50.3 36.2	46.3 37.7	62 8.5	47.4 36.5	49.7 36.2	48.4 35.5	0.463	0.893	0.856
Normal AST (U/L)	N %	43 59.70%	15 57.70%	0 0%	15 53.60%	101 87.10%	15 12.90%	0.31	0.576	0.336
High AST (U/L)	N %	29 40.30%	11 42.30%	2 100%	13 46.40%	69 82.10%	15 17.90%			

Citation: Waly F, El-Sayed Zaki M, Shabana H, et al. Single nucleotide polymorphism of the promotor region of Interleukin-10 gene did not show significant association with spontaneous clearance of hepatitis C virus infection in Egyptian patients. *Gastroenterol Hepatol Open Access*. 2023;14(6):181–189. DOI: 10.15406/ghoa.2023.14.00565

Table 7 Continued...

CHC		AA N=72	AG N=26	GG N=2	AG+GG N=28	A N=170	G N=30	P1	P2	P3
ALT (U/L)	Mean ±SD	46.1 28	48.9 47.4	76.5 19.1	50.8 46.4	46.5 31.5	52.5 45.4	0.24	0.794	0.369
Normal ALT (U/L)	N %	44 61.10%	16 61.50%	0 0%	16 57.10%	104 86.70%	16 13.30%	0.294	0.716	0.419
High ALT (U/L)	N %	288 3.90%	10 38.50%	2 100%	12 42.90%	66 82.50%	14 17.50%			
FIB4	Mean ±SD	3 3.3	2.6 2.1	2.6 0	2.6 2	3	2.6 2	0.829	0.721	0.571
Albumin (mg/dl)	Mean ±SD	4.2 0.4	4 0.7	4.5 0.2	4 0.7	4.2 0.5	4.1 0.7	0.169	0.154	0.304
Bilirubin (mg/dL)	Mean ±SD	0.8 0.2	0.8 0.1	0.8 0.2	0.7 0.2	0.8 0.3	0.8 0.3	0.999	0.985	0.978
Creatinine (mg/dl)	Mean ±SD	0.9 0.2	0.9 0.2	0.7 0.1	0.9 0.2	0.9 0.24	0.9 0.21	0.577	0.708	0.925
VL (iu/ml)	Median Range	622910.5 6500-800114000	605729.5 20207-31300000	80524.5 69502-91547	461190 20207-31300000	605729.5 6500-800114000	342199.5 20207-31300000	0.187	0.45	0.241
Low VL	N %	11 15.30%	4 15.40%	2 100.00%	6 21.40%	26 15.30%	8 26.70%			
Moderate VL	N %	26 36.10%	10 38.50%	0 0.00%	10 35.70%	62 36.50%	10 33.30%	0.154	0.768	0.305
High VL	N %	35 48.60%	12 46.20%	0 0.00%	12 42.90%	82 48.20%	12 40.00%			

P1= comparison between AA, AG GG; P2 = comparison between AG+GG versus AA; P3= comparison between alleles.

Table 8 Comparison of clinical and radiologic data of IL10 (-1082 A/G) genotypes and alleles in SVC group

SVC		AA N=31	AG N=17	GG N=2	AG+GG N=19	A N=79	G N=21	P1	P2	P3
Age (years)	Mean ±SD	54 8.5	51.4 8.6	44.5 7.7	50.6 8.6	53.5 8.5	50.1 8.6	0.237	0.186	0.11
Males	N %	16 51.6	7 41.2	1 50	8 42.1	39 49.4	9 42.9	0.772	0.514	0.596
Females	N %	15 48.4	10 58.8	1 50	11 57.9	40 50.6	12 57.1			
Weight (kg)	Mean ±SD	89.1 13	86.3 14.7	89 11.3	86.6 14.1	88.5 13.3	86.9 13.7	0.794	0.528	0.611
Height (cm)	Mean ±SD	167.6 8.7	165.65 5.219	174.5 4.9	166.5 5.7	167.2 8	167.3 6.1	0.275	0.629	0.961
BMI(Kg/m2)	Mean ±SD	31.6 3.9	31.3 4.7	29.1 2.1	3.9 4.5	31.6 4.1	31 4.4	0.71	0.664	0.519
Smoking	N %	8 25.8	5 29.4	0 0	5 26.3	21 26.6	5 23.8	0.668	0.968	0.797
DM	N %	6 19.4	1 5.9	0 0	1 5.3	13 16.5	1 4.8	0.554	0.229	0.289

P1=comparison between AA, AG GG; P2= comparison between AG + GG versus AA; P3= comparison between alleles.

Table 9 Comparison of laboratory data of IL10 (-1082 A/G) genotypes and alleles in SVC group

SVC		AA N=31	AG N=17	GG N=2	AG+GG N=19	A N=79	G N=21	P1	P2	P3
TLC (×109/L)	Mean ±SD	5.8 1.5	5.3 1.73	6.2 0.4	5.4 1.6	5.7 1.6	5.5 1.6	0.58	0.43	0.607
Neutrophil (%)	Mean ±SD	47.3 12.1	47.7 10.8	49 2.8	47.9 10.2	47.4 11.7	48 9.8	0.977	0.872	0.842
Lymphocytes (%)	Mean ±SD	40.9 10.9	39.3 8.2	48.5 0.7	40.3 8.3	40.6 10.3	41 8.3	0.46	0.819	0.849

Citation: Waly F, El-Sayed Zaki M, Shabana H, et al. Single nucleotide polymorphism of the promotor region of Interleukin-10 gene did not show significant association with spontaneous clearance of hepatitis C virus infection in Egyptian patients. *Gastroenterol Hepatol Open Access*. 2023;14(6):181–189. DOI: 10.15406/ghoa.2023.14.00565

Table 9 Continued...

SVC		AA N=31	AG N=17	GG N=2	AG+GG N=19	A N=79	G N=21	P1	P2	P3
N/L ratio	Mean ±SD	1.5 0.8	1.5 0.8	1 0.1	1.4 0.7	1.5 0.8	1.3 0.7	0.716	0.807	0.544
Hemoglobin (g/dl)	Mean ±SD	12.6 1.4	12.2 1.7	11.4 1.5	12.2 1.6	12.6 1.5	12.1 1.7	0.476	0.322	0.242
Platelets (×109/L)	Mean ±SD	224.5 71.5	210.3 61.8	238.5 58.6	213.3 60.6	221.5 69	215.7 59.5	0.734	0.573	0.728
INR	Mean ±SD	1.2 0.1	1.1 0.1	1 0.01	1.2 0.1	1.1 0.1	1.1 0.1	0.564	0.959	0.759
Glucose (mg/dl)	Mean ±SD	121.8 42.6	115.2 30.4	121.5 16.3	115.9 29	120.4 39.9	116.4 27.8	0.654	0.992	0.672
AST (U/L)	Mean ±SD	28.5 14.6	24.6 9.4	33 15.6	25.5 9.9	27.6 13.6	26.2 10.3	0.664	0.794	0.655
Normal AST(U/L)	N %	25 80.60%	15 88.20%	1 50%	16 84.20%	65 79.30%	17 20.70%	0.403	0.75	0.888
High AST(U/L)	N %	6 19.40%	2 11.80%	1 50%	3 15.80%	14 77.80%	4 22.20%			
ALT (U/L)	Mean ±SD	31.1 15.4	30.5 12.9	34 2.8	30.9 12.2	31 14.7	31.2 11.6	0.681	0.653	0.956
Normal ALT(U/L)	N %	25 80.60%	15 88.20%	2 100%	17 89.50%	65 77.40%	19 22.60%	0.785	0.693	0.511
High ALT(U/L)	N %	6 19.40%	2 11.80%	0 0%	2 10.50%	14 87.50%	2 12.50%			
FIB4 score	Mean ±SD	1.3 0.4	1.3 0.6	1.2 1	1.3 0.6	1.3 0.5	1.3 0.6	0.683	0.384	0.825
Albumin (g/dl)	Mean ±SD	4.2 0.4	4.2 0.3	4.4 0.4	4.3 0.3	4.3 0.4	4.3 0.4	0.857	0.658	0.595
Bilirubin (mg/dl)	Mean ±SD	0.6 0.1	0.8 0.6	0.5 0.1	0.7 0.5	0.7 0.3	0.7 0.6	0.442	0.323	0.509
Creatinine (mg/dL)	Mean ±SD	0.8 0.2	0.8 0.1	0.7 0.1	0.8 0.2	0.9 0.2	0.8 0.2	0.287	0.206	0.132

P1= comparison between AA, AG GG; P2= comparison between AG+GG versus AA; P3= comparison between alleles

Table 10 Logistic regression analysis for prediction of SVC

	Univariable				Multivariable			
	p	OR	95% CI		*p*	OR	95% CI	
Gender	0.762	0.926	0.563	1.522				
BMI(Kg/m^2)	0.282	0.965	0.903	1.03				
N/L ratio	0.79	0.966	0.748	1.247				
INR	0.804	1.403	0.097	2.293				
AST(U/L)	<0.001	0.956	0.932	0.98	0.172	0.982	0.958	1.008
ALT(U/L)	0.002	0.969	0.95	0.989	0.607	1.006	0.985	1.027
FIB4 score	0.006	0.64	0.464	0.882	0.049	0.718	0.515	0.999
IL10 (AG+GG)	0.216	1.322	0.849	2.058				
IL10 alleles	0.192	1.506	0.812	2.795				

Table 11 Logistic regression analysis for prediction of CHC

	Univariable				Multivariable			
	P	OR	95% CI		*p*	OR	95% CI	
Gender	0.762	1.08	0.657	1.776				
BMI(Kg/m^2)	0.282	1.037	0.971	1.107				
N/L ratio	0.79	1.04	0.804	1.344				
INR	0.804	0.835	0.06	11.57				
AST(U/L)	<0.001	1.018	1.004	1.033	0.525	1.008	0.983	1.034
ALT(U/L)	0.002	1.012	1	1.025	0.624	0.995	0.973	1.016
FIB4 score	0.006	1.573	1.139	2.174	0.042	1.487	0.981	2.256
IL10 (AG+GG)	0.216	0.756	0.542	1.608				
IL10 alleles	0.192	0.664	0.358	1.232				

Citation: Waly F, El-Sayed Zaki M, Shabana H, et al. Single nucleotide polymorphism of the promotor region of Interleukin-10 gene did not show significant association with spontaneous clearance of hepatitis C virus infection in Egyptian patients. *Gastroenterol Hepatol Open Access*. 2023;14(6):181–189. DOI: 10.15406/ghoa.2023.14.00565

Discussion

The outcome of HCV infection may be affected by many factors including mode of transmission, alcohol intake, gender, viral strain, coinfection with other viruses and host immune response. The impact of the cytokine gene polymorphisms on the pathogenesis of HCV infection has also been investigated.[11] IL-10 is an immunoregulatory cytokine produced by Th2 cells, monocytes, macrophages, and regulatory T cells. It negatively regulates the response of Th1 lymphocytes and suppresses the action of pro-inflammatory cytokines such as IL-2, TNF-α & IFN-γ. On the other hand, it has antifibrogenic effect.[12] Its production and plasma levels vary among individuals due to three single-nucleotide polymorphisms (SNPs) (-1082 G/A, -819 C/T and -592 C/A) in the promoter region of the IL-10 gene. IL-10 polymorphic variants have been found to play an important role in the outcome of HCV infection.[13] Individuals who are homozygous for IL-10 AA at position (-1082) have significantly lower levels of plasma IL-10 levels with better ability to spontaneously clear HCV infection. Individuals who show (-1082) G/G genotype have been shown to produce two fold greater quantities of IL-10 when compared to individuals exhibiting the A/A or G/A genotypes with less ability to clear the virus.[9]

The present study found that IL10 rs1800896 AA genotype (major genotype) showed frequency of 72% in CHC group and 62% in SVC group. The alternative genotype (GG) showed frequency of 2% in CHC group and 4% in SVC group. AG genotype showed frequency of 26% in CHC group and 34% in SVC group. Dominant model (AG+GG) showed frequency of 28% in CHC group and 38% in SVC group. Calculation of allele frequency in the studied groups revealed that the major allele (A) showed frequency of 85% in CHC group and 79% in SVC group, while alternative or minor allele (G) showed frequency of 15% in CHC group and 21% in SVC group. Taking AA as the reference genotype and A as the reference allele; IL-10 (rs1800896) genotypes and alleles did not show significant association with SVC. Earlier studies concerning IL-10 promoter (-1082) polymorphism rs1800896 and HCV infection outcome were controversial. A study reported an association of the GG genotype with persistent infection and AG genotype with self-limiting infection.[14] Another study revealed an association of the GG genotype with persistent infection, although this was based on a comparison with healthy controls.[11] It was reported that that AG genotype was higher in controls than in HCV patients, while genotype GG was higher in CHC patients.[9] This was explained by the mechanism that GG genotype was associated with high IL10 production with downregulation of Th1 response, resulting in failure of HCV eradication and perpetuation of viral infection.[15]

On the contrary, a study showed that GG genotype was highest in patients with SVC compared to those with CHC, suggesting that an increased production of IL-10 was protective against CHC.[16] Also, Ramos et al. suggested that the AA genotype was more frequently observed in patients with CHC, while GG genotype was more frequently seen in those with SVC. They claimed that IL-10 may have anti-fibrotic properties that could lead to a slow progression of liver disease.[17] It was found that there was no significant association between polymorphisms in the IL-10 (-1082, -819) or cytokine haplotype and either HCV clearance or severity of hepatitis.[18] Another study conducted in England revealed that IL-10 (-1082) genotype did not contribute to HCV clearance. An Egyptian study comparing CHC and SVC patients found no significant differences regarding both the genotypes and alleles of IL-10 (-1082) rs1800896 gene.[19]

Another Egyptian study reported that the distribution of the different IL-10 rs1800896 genotypes was similar in the healthy controls and HCV patients.[13] These conflicting results could be due to several factors such as differences in sample size, genetic heterogeneity of various populations and different gene–gene or gene–environment interactions. It could be also due to differences in ethnic groups of patients and differences in distribution of hepatitis C virus genotypes among population that might affect the outcome of the infection.[20] The present study showed that there were no significant differences in clinical and radiologic data regarding IL-10 (-1082 A/G) genotypes and alleles in either CHC or SVC groups. Similarly, another study did not find association between the (-1082A/G) polymorphism and liver fibrosis aggressiveness or cirrhosis in the CHC patients.[15] Conversely, some authors demonstrated that the IL-10 (-1082) AA genotype was associated with an increased risk of chronic hepatitis and cirrhosis, while IL-10 (-1082) AG genotype was associated with a reduced risk. They also reported that IL-10 (-1082) GG genotype with high IL10 production was associated with lower risk of progression to cirrhosis due to potent anti-inflammatory and modulatory effect of IL10 on hepatic fibrogenesis.[9] Regarding CHC group**,** no significant differences were found regarding the laboratory data, including ALT, AST and viral load between IL-10 (-1082 A/G) genotypes and alleles**.** However, some investigators found that IL-10 (-1082) AA and -1082 A were associated with an increased risk of HCV RNA replication, while IL-10 (-1082) AG and -1082 G were associated with a reduced risk.[21]

A study analysis of the role of allelic or genotype variations of IL-10 and its association with hepatocellular injury, as tested by ALT, reported that there was no significant difference in the IL-10 (-1082) genotype profile between patients with normal and patients with elevated ALT levels.[22] In the present study, CHC patients showed significantly higher FIB4 than SVC subjects. It was reported that the mean HAI and fibrosis scores of the PCR negative women were also significantly lower than those of PCR positive women (p=0.000).[23] Also, the present study showed that CHC patients had significantly lower PLC than SVC subjects. This could be explained by the presence of hypersplenism, decreased platelet production due to decreased hepatic thrombopoietin (TPO) secretion and CHC induced bone marrow suppression.[24] Logistic regression analysis for prediction of SVC using gender, BMI, N/L ratio, INR, AST, ALT, FIB4 and IL10 genotypes and alleles, as covariates, showed that lower ALT, AST and FIB4 were associated with SVC in univariable analysis.

On multivariate analysis, only lower FIB4 was considered a predictor of SVC. A study revealed that SVC tended to be high in patients with high ALT levels which could be a reflection of the immune response to acute HCV infection. However, there was no correlation between SVC and serum AST.[25] However, another study did not find an association between SVC and ALT level. Lack of association with these factors could be related to the lack of exact date of high-risk exposure to infection.[26] A third study found no association of SVC with age, sex, race & peak ALT level.[27] The limitation of the present study was the small sample size. Also, serum level of IL-10 was not measured in the different genotypes of the IL-10 gene. Large scale studies in other areas of Egypt are needed to confirm our result. Also, the concurrent determination of the serum level of IL10 and estimation of its expression pattern is needed. Studying of other IL10 SNPs (-819 T/C; -592 A/C) and exploring their role in predicting SVC should be done. Studying of other cytokine gene polymorphisms especially the IL-28B, IL-4(+33); TNF-α (−238 G/A and −308 G/A), IL-4 (+33C/T) & IFN-γ (+874 T/A), is needed to explore their role in predicting SVC of HCV infection.

Conclusion

Single nucleotide polymorphism of the promoter region of the IL-10 gene, 1082 (G/A; rs1800896), did not show significant association

Citation: Waly F, El-Sayed Zaki M, Shabana H, et al. Single nucleotide polymorphism of the promotor region of Interleukin-10 gene did not show significant association with spontaneous clearance of hepatitis C virus infection in Egyptian patients. *Gastroenterol Hepatol Open Access.* 2023;14(6):181–189.
DOI: 10.15406/ghoa.2023.14.00565

with the spontaneous clearance of hepatitis C virus infection in Egyptian patients. In chronic hepatitis C patients, this SNP had no significant association with the serum HCV viral load, CTP class, PLC or FIB4 score. FIB4 score may predict the SVC of HCV infection.

Acknowledgments

None.

Conflicts of interest

The authors declare no conflicts of interest.

References

1. Elgharably A, Gomaa AI, Crossey MM, et al. Hepatitis C in Egypt - past, present, and future. *International Journal of General Medicine*. 2017;10:1–6.
2. Amer F, Gohar M, Yousef M. Epidemiology of Hepatitis C virus infection in Egypt. *International Journal of Tropical Disease & Health*. 2015;7(3):119–131.
3. El-Serag HB. Epidemiology of viral hepatitis and hepatocellular carcinoma. *Gastroenterology*. 2012;142(6):1264–1273.e1.
4. Huang, H, Duggal P, Thio CL, et al. Fine-mapping of genetic loci driving spontaneous clearance of hepatitis C virus infection. *Scientific Reports*. 2017;7(1):15843.
5. Taherkhani R, Farshadpour F. Global elimination of hepatitis C virus infection: Progresses and the remaining challenges. *World Journal of Hepatology*. 2017;9(33):1239–1252.
6. Jordovic J, Simonovic-Babic J, Gasic V, et al. IL-28B genotypes as predictors of long-term outcome in patients with hepatitis C-related severe liver injury. *The Journal of Infection in Developing Countries*. 2019;13(6):526–535.
7. Moreira ST, Giovanni FS, Camila FV, et al. Influence of cytokine and cytokine receptor gene polymorphisms on the degree of liver damage in patients with chronic hepatitis C. *Meta Gene*. 2016;9:90–96.
8. McLane LM, Abdel-Hakeem MS, Wherry EJ. CD8 T cell exhaustion during chronic viral infection and cancer. *Annual Review of Immunology*. 2019;37(1):457–495.
9. Sheneef A, Esmat MM, Mohammad AN, et al. Interleukin-10 and interferon gamma gene polymorphisms and Hepatitis C virus–related liver cirrhosis risk. *Journal of Interferon & Cytokine Research*. 2017;37(4):175–180.
10. Martínez SM, Crespo G, Navasa M, et al. Noninvasive assessment of liver fibrosis. *Hepatology*. 2011;53(1):325–335.
11. da Silva NMO, Germano FN, Vidales-Braz BM, et al. Polymorphisms of IL-10 gene in patients infected with HCV under antiviral treatment in southern Brazil. *Cytokine*. 2015;73(2):253–257.
12. Ouyang W, O'Garra A. IL-10 Family Cytokines IL-10 and IL-22: from Basic Science to Clinical Translation. *Immunity*. 2019;50(4):871–891.
13. Abdelraheem WM, Hassuna NA, Abuloyoun SM, et al. Interleukin-10. rs1800896 and Interleukin-18.rs1946518 gene polymorphisms could not predict the outcome of hepatitis C virus infection in Egyptian patients treated with pegylated interferon plus ribavirin. *Archives of Virology*. 2016;161(9):2473–2480.
14. Świątek-Kościelna B, Kałużna E, Strauss E, et al. Interleukin 10 gene single nucleotide polymorphisms in Polish patients with chronic hepatitis C: Analysis of association with severity of disease and treatment outcome. *Human Immunology*. 2017;78(2):192–200.
15. Guo PF, Jin J, Sun X. Influence of IL10 gene polymorphisms on the severity of liver fibrosis and susceptibility to liver cirrhosis in HBV/HCV-infected patients. *Infection Genetics and Evolution*. 2015;30:89–95.
16. Khan AJ, Saraswat VA, Choudhuri G, et al. Association of interleukin-10 polymorphisms with chronic hepatitis C virus infection in a case-control study and its effect on the response to combined pegylated interferon/ribavirin therapy. *Epidemiol Infect*. 2015;143(1):71–80.
17. Ramos, J, Silva R, Hoffmann L, et al. Association of IL-10, IL-4, and IL-28B gene polymorphisms with spontaneous clearance of hepatitis C virus in a population from Rio de Janeiro. *BMC Research Notes*. 2012;5(1):508.
18. Sun XR, Wu J, Shi KQ, et al. Relationship between IL-10 gene −1082A/G and −592C/A polymorphisms and the risk of hepatitis C infection: a meta-analysis. *Journal of Viral Hepatitis*. 2013;20(9):602–611.
19. Helal SF, Gomaa HE, Thabet EH, et al. Impact of IL-10 (-1082) promoter–single nucleotide polymorphism on the outcome of Hepatitis C virus genotype 4 infection. *Clinical Medicine Insights: Gastroenterology*. 2014;7:19–24.
20. Yang R, Yang X, Xiu B, et al. Hepatitis C virus genotype analyses in chronic hepatitis c patients and individuals with spontaneous virus clearance using a newly developed serotyping assay. *Journal of Clinical Laboratory Analysis*. 2017;31(1):e22014.
21. Gao QJ, Xie JX, Wang LM, et al. Interaction effects among IFN-γ+874, IL-2-330, IL-10-1082, IL-10-592 and IL-4-589 polymorphisms on the clinical progression of subjects infected with hepatitis B virus and/or hepatitis C virus: a retrospective nested case–control study. *BMJ Open*. 2017;7(8):e013279.
22. Li J, Liu Y, Xu F, et al. Three polymorphisms in the IL-10 gene and the risk of HCV infection: a meta-analysis plus a Chinese association study involving 1140 subjects. *Epidemiology and Infection*. 2013;141(5):893–904.
23. Tabll AA, Marwa A kaddos, Aymn Abbas, et al. Association between serum aminotransferase enzymes–lipid profile ratio and spontaneous HCV clearance in blood donors. *Future Virology*. 2016;11(1):19–29.
24. Mitchell O, Feldman DM, Diakow M, et al. The pathophysiology of thrombocytopenia in chronic liver disease. *Hepatic Medicine*. 2016;8:39–50.
25. Cho YK, Kim YN, Song BC. Predictors of spontaneous viral clearance and outcomes of acute hepatitis C infection. *Clinical and Molecular Hepatology*. 2014;20(4):368–375.
26. Hashem M, Zaghla H, Zakaria Z, et al. High spontaneous clearance of symptomatic iatrogenic acute hepatitis C genotype 4 infection. *Journal of Medical Virology*. 2018;90(12):1841–1847.
27. Tsertsvadze T, Sharvadze L, Chkhartishvili N, et al. The natural history of recent hepatitis C virus infection among blood donors and injection drug users in the country of Georgia. *Virology Journal*. 2016;13(1):22.

Citation: Waly F, El-Sayed Zaki M, Shabana H, et al. Single nucleotide polymorphism of the promotor region of Interleukin-10 gene did not show significant association with spontaneous clearance of hepatitis C virus infection in Egyptian patients. *Gastroenterol Hepatol Open Access*. 2023;14(6):181–189.
DOI: 10.15406/ghoa.2023.14.00565