

ORIGINAL ARTICLE

Impact of genetic polymorphisms of four cytokine genes on treatment induced viral clearance in HCV infected Egyptian patients



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IL-10;
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IFN- γ ;
TGF- β

Abstract *Background:* Many factors contribute for viral clearance and response to antiviral therapy. Genetic polymorphisms of cytokines, chemokines, and their receptors can alter the immune response against Hepatitis C virus (HCV).

Aim of the study: The aim of the current study is to assess single nucleotide polymorphism (SNP) in the promoter region of IL-10, TNF- α , IFN- γ and TGF- β as predictors of response to combined Pegylated interferon α /ribavirin (PEG-IFN/RBV) therapy in chronic HCV infected Egyptian patients.

Patients and methods: The study was conducted on 150 HCV infected patients and 100 apparently healthy control subjects. All patients were treated with PEG-IFN/RBV. They were classified according to their response to treatment.

Genotyping of IL-10, TNF- α , IFN- γ and TGF- β were performed on peripheral blood DNA using polymerase chain reaction–restriction fragment-length polymorphism (PCR-RFLP) and primer specific assays.

Results: Overall, 83/150 (55.3%) patients achieved sustained virological response (SVR), whereas 67 (44.7%) did not. Age and BMI were significantly lower in patients who achieved SVR ($P < 0.05$). IL-10 at site (–1082) GG genotype was associated with SVR where odds ratio was 1.98 with 95% confidence interval (1.34–3.65). None of the other genes showed a significant association with SVR.

Conclusion: Analysis of IL-10 SNP at promoter site (–1082) could be used as a pretreatment predictor of response to combined PEG-IFN/RBV treatment.

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1. Introduction

Hepatitis C virus (HCV) infection is an important public health problem, which affects more than 170 million persons worldwide [1]. The long-term impact of HCV infection is highly variable, ranging from minimal histological changes to extensive fibrosis and cirrhosis or hepatocellular carcinoma (HCC) [2]. Interferon had been the cornerstone of HCV therapy for almost two decades [3]. Direct-acting anti-viral (DAA) drugs active against different targets of HCV became available. These potent anti-HCV drugs may be combined with Pegylated interferon α /ribavirin (PEG-IFN/RBV) according to recent guidelines [4,5]. Attempts to identify factors predicting favorable response to therapy showed that sustained virological response (SVR) rates with PEG-IFN/RBV are dependent on a multitude of host and viral factors. However, current data on genotype 4 (G4) chronic hepatitis C (CHC) regarding treatment responsiveness and duration are limited and controversial [6].

A strong natural killer (NK) cell and T helper 1 (Th1) cell-mediated immune response seems to be a key factor in the protection against HCV infection [7]. In addition, viral persistence and a deficient response to antiviral therapy have been associated with the production of inappropriate levels of cytokines [8]. Interleukin 10 (IL-10) is a multifunctional cytokine produced by macrophages, monocytes, and T-helper cells that down regulates the synthesis of pro-inflammatory cytokines and has a modulatory effect on hepatic fibrogenesis [9]. Heterogeneity in the promoter region of the IL-10 gene has been reported to have a role in determining the initial and sustained response of chronic hepatitis C to IFN- α therapy [10].

Tumor necrosis factor alpha (TNF- α) may play a role in the pathogenesis of acute and chronic HCV infection. Some genetic polymorphisms in the human TNF- α promoter region, such as the G-to-A transition at positions +308 have been shown to influence TNF- α expression [11].

Interferon gamma (IFN- γ) is critical for host defense against a variety of intracellular pathogens, including HCV [12]. It has been established that the T-to-A polymorphism at position +874 of the first intron of IFN- γ gene could directly influence IFN- γ production [13].

Transforming growth factor- β (TGF- β) also can cause dysregulation of host immune response in chronic HCV patients [14].

The aim of the work is to assess single nucleotide polymorphism (SNP) in the promoter region of IL-10 at sites (-1082 G/A, -819 C/T and -592 C/A), TNF- α (-308 G/A), IFN- γ (+874 A/T) and TGF- β (+869 T/C and +915 G/C) as predictors of response to antiviral therapy using PEG-IFN/RBV therapy in Egyptian patients with CHC.

1.1. Patients and methods

This study included 150 consecutive chronic HCV infected adult patients who were recruited from the National Liver Institute (NLI), Menoufia University, Egypt in the period from September 2010 to June 2011. In addition, 100 apparently healthy subjects of matched age and gender served as the control group. All patients received antiviral treatment with follow up protocol according to the standard clinical practice for 18 months. The study was approved by the Institution's ethics

committee, and a written informed consent was taken from all participants enrolled in the study. The work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments in humans.

Patients had to meet the following criteria:

(1) Treatment naïve patients. (2) They had positive HCV RNA, higher than 1000 IU/mL. (3) Liver biopsy was done within 3 months before initiation of therapy. (4) Age is not less than 18 years. (5) Normal thyroid and kidney functions. (6) No evidence of decompensated liver disease or hepatocellular carcinoma. (7) No evidence of other etiology of liver disease. (8) No significant history of cardiovascular or neuropsychiatric diseases.

All patients received PEG-IFN α -2a administered subcutaneously at a dose of 180 μ g per week or PEG-IFN α -2b administered subcutaneously at a dose of 1.5 μ g/kg/week and twice-daily RBV dosed orally according to body weight (< 75 kg, 1000 mg daily; \geq 75 kg, 1200 mg daily). Patients who failed to achieve EVR (>2 log₁₀ decrease in HCV RNA at Week 12) or Non-detectable HCV RNA at Week 24 discontinued all therapy. Those who completed 48 weeks of therapy were followed up for 24 weeks.

Sustained virological response (SVR) was defined as undetectable levels of HCV RNA 24 weeks after the completion of therapy; relapse was defined as detectable HCV RNA during follow up in patients who had undetectable HCV RNA at the end of treatment; non-response was defined as detectable HCV RNA at the end of treatment.

1.2. Laboratory investigations

Baseline blood samples were obtained before commencing treatment and were analyzed for blood chemistry, HCV RNA and hematology, using fully automated auto analyzer SYNCHRON CX9ALX (Beckman Coulter Inc., CA, USA), COBAS® TaqMan® HCV assay (Roche Molecular Diagnostics, CA, USA) with lower limit of quantitation of 15 IU/mL, and Sysmex K-21 (Sysmex Corporation, Kobe, Japan), respectively. Periodic blood samples were taken from patients only for determination of transaminases, complete blood count and HCV RNA on weeks (12, 24, and 48) during treatment and at 6 months after end of treatment.

The histopathological assessment of necro-inflammatory grade and fibrosis stage were done using the modified Ishak scoring system before commencing treatment [15].

1.3. Molecular testing

Genomic DNA was extracted from EDTA-treated peripheral blood using Gene JET Whole Blood Genomic DNA Purification Mini Kit, (Thermo Fisher Scientific, MA, USA) (see Table 1).

1.4. General protocol for PCR-RFLP genotyping

Reaction master mix for amplification constituted of 50 pmol of each of forward and reverse primers; 12.5 μ l of 2 \times Dream Taq Green PCR Master Mix (Thermo Fisher Scientific, MA, USA) and 100 ng of the genomic DNA and the total volume of reaction was topped up to 25 μ l with DNase-free water.

Table 1 Primers sequences.

	Primers sequence	Genotyping method	Restriction enzyme
IL-10-1082	Forward: 5'-TCTGAAGAAGTCTGATGTCACTG-3' Reverse: 5'-ACTTTCATCTTACCTATCCCTACTTCC-3'	PCR-RFLP	MnII
-819	Common primer: 5'-AGGATGTGTTCCAGGCTCCT-3' C primer: 5'-CCCTTGACACGTGATGTAAC-3' T primer: 5'-ACCCTTGACAGGTGATGTAAT-3'	Primer specific	-
-592	Forward: 5'-CCTAGGTCACAGTGACGTGG-3' Reverse: 5'-GGTGAGCACTACCTGACTAGC-3'	PCR-RFLP	RsaI
TNF- α (-308)	Forward: 5' AGGCAATAGGTTITGAGGGCCAT-3' Reverse: 5' TCCTCCCTGCTCCGATTCCG-3'	PCR-RFLP	NcoI
INF- γ (+874)	Forward: 5'-TCAACAAAGCTGATACTCCA-3' (A) Reverse: 5'-TTCTTACAACACAAAATCAAATCA-3' (T) Reverse: 5'-TTCTTACAACACAAAATCAAATCT-3'	Primer specific	-
TGF- β (+869) (+915)	Forward: 5'-GTTATTTCCGTGGATACTGAGAC-3' Reverse: 5'-GACCTCTTGCGTAGTAGTCG-3'	PCR-RFLP	MspAII BglI

The PCR was performed on Applied Biosystems Veriti™ thermocycler (Thermo Fisher Scientific Inc., Life Technologies™, CA, USA).

After confirmation of successful PCR amplification using 1.5% agarose gel electrophoresis, 10 μ l of the amplification product was added to a digestion mixture of 3 μ l buffer (R) [10 mM Tris-HCl (pH 8.5), 10 mM MgCl₂, 100 mM KCl, 0.1 mg/ml BSA], 10 units of an appropriate restriction enzyme and the volume was topped up with H₂O to 30 μ l and incubated at 37 °C overnight. The restriction digests were electrophoresed on 2% Agarose gels (Axygen, CA, USA) for 3 h at 60 V and photographed using InGenius gel documentation system (Syngene, Cambridge, UK).

1.4.1. Analysis of SNP in the promoter region of the IL-10 at sites (-1082, -819 and -592)

1.4.1.1. *IL-10* (-1082 G/A) (*rs1800896*). Genotyping was done using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay as previously reported [16,17]. An amplification product 196 bp was obtained using standard PCR assay, according to cycling conditions consisted of an initial denaturation step at 94 °C for 2 min, followed by 40 cycles of 94 °C for 30 s, 59 °C for 55 s and 72 °C for 45 s, with a final extension of 72 °C for 5 min. The product was digested using MnII (Thermo Fisher Scientific, MA, USA) (see Fig. 1).

1.4.1.2. *IL-10* (-819 C/T) (*rs1800871*). Genotyping was performed using primer specific polymerase chain reaction assay as previously reported [18,19].

A 233-bp fragment spanning position -819 was amplified using primers with concentration 35 pmol for each primer.

Reaction mix constituted of 35 pmol of each of forward and reverse primers; 12.5 μ l of 2 \times Dream Taq Green PCR Master Mix (Thermo Fisher Scientific, MA, USA) and 100 ng of the genomic DNA and the total volume of reaction was topped up to 25 μ l with DNase-free water. The PCR was performed on Applied Biosystems Veriti™ thermocycler (Thermo Fisher Scientific Inc., Life Technologies™, CA, USA) according to cycling conditions consisting of an initial

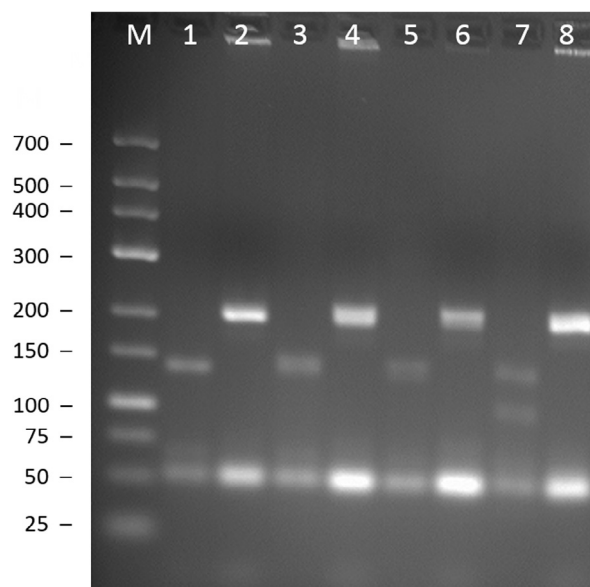


Figure 1 Agarose gel electrophoresis of PCR-RFLP for IL-10 (-1082 G/A) genotypes analysis. Digested and undigested samples were electrophoresed against a ready-to-use, Gene Ruler™ low range DNA ladder 25–700 bp (Thermo Fisher Scientific, CA, USA), landmark bands are 100 bp and 300 bp respectively. PCR product of 196 bp for the undigested samples in lanes 2, 4, 6 and 8, genotyping was done using restriction enzyme MnII. Lanes 1, 3 and 5 show G allele with fragments at 58 and 110 bp (fragment 28 was not visualized), while lane 7 shows A with 58 and 138 bp as well as G allele at 58 bp and 110 bp.

denaturation step at 95 °C for 3 min, followed by 5 cycles of 94 °C for 45 s, 70 °C for 45 s and 72 °C for 20 s, followed by 25 cycles of 95 °C for 25 s, 65 °C for 50 s and 72 °C for 45 s followed by 5 cycles of 95 °C for 25 s, 55 °C for 45 s followed by a final extension of 72 °C for 2 min. The PCR product of 233 bp was visualized on the 2% agarose gel (see Fig. 2).

1.4.1.3. *IL-10* (−592 C/A) (*rs1800872*). Genotyping was done using polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) assay as previously reported [17].

Amplification of 412-bp fragment spanning position −592 was obtained using standard PCR assay, according to cycling conditions consisted of an initial denaturation step at 94 °C for 2 min, followed by 40 cycles of 94 °C for 30 s, 59 °C for 55 s and 72 °C for 45 s, with a final extension of 72 °C for 5 min. The product was then digested using *RsaI* (Thermo Fisher Scientific, MA, USA) (see Fig. 3).

1.4.2. *TNF-α* gene polymorphism (−308 G/A) (*rs1800629*)

Genotyping was done using polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) assay as previously reported [19]

An amplification product 107 bp was obtained using standard PCR assay, according to cycling conditions consisted of an initial denaturation step at 94 °C for 3 min, followed by 40 cycles of 94 °C for 30 s, 49 °C for 45 s and 72 °C for 45 s, with a final extension of 72 °C for 5 min. The product was then digested using restriction enzyme *NcoI* (Thermo Fisher Scientific, MA, USA) (see Fig. 4).

1.4.3. *INF-γ* gene polymorphism (+874 A/T) (*rs2430561*)

Genotyping was performed using primer specific polymerase chain reaction assay as previously reported [20] where a 261-bp fragment spanning position +874 was amplified.

Reaction mix constituted of 50 pmol of each of forward and reverse primers; 12.5 μl of 2× Dream Taq Green PCR Master Mix (Thermo Fisher Scientific, MA, USA) and 100 ng of the genomic DNA and the total volume of reaction

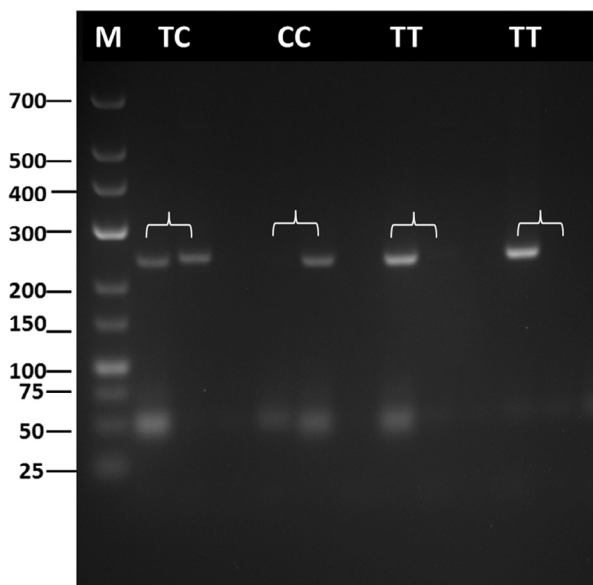


Figure 2 Agarose gel electrophoresis of primer specific PCR for *IL-10* (−819 C/T) genotypes analysis. Four samples were electrophoresed against a ready-to-use, Gene Ruler™ low range DNA ladder 25–700 bp (Thermo Fisher Scientific, CA, USA), landmark bands are 100 bp and 300 bp respectively. PCR product of 233 bp for the two specific primers for each sample was run in two consecutive lanes and genotypes were labeled on the gel.

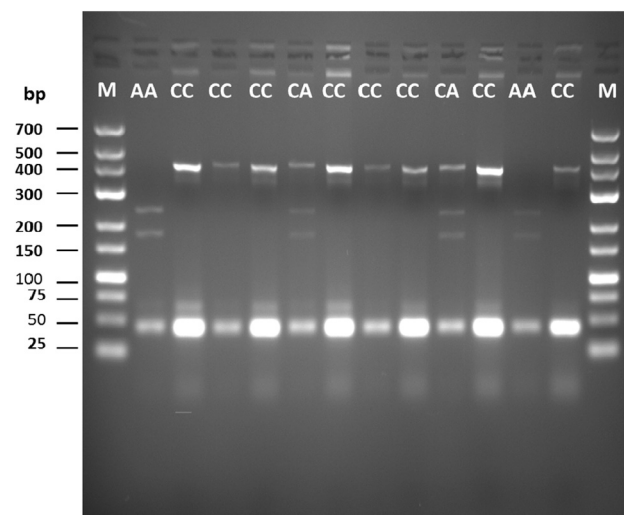


Figure 3 Agarose gel electrophoresis of PCR–RFLP fragments for *IL10* (−592 C/A) genotypes analysis. The C allele was identified by the presence of 412-bp fragment. The A allele had an *RsaI* site yielded a 176 bp and 236-bp fragment only. The samples were electrophoresed against a ready-to-use, Gene Ruler™ low range DNA ladder 25–700 bp (Thermo Fisher Scientific, CA, USA), landmark bands are 100 bp and 300 bp respectively.

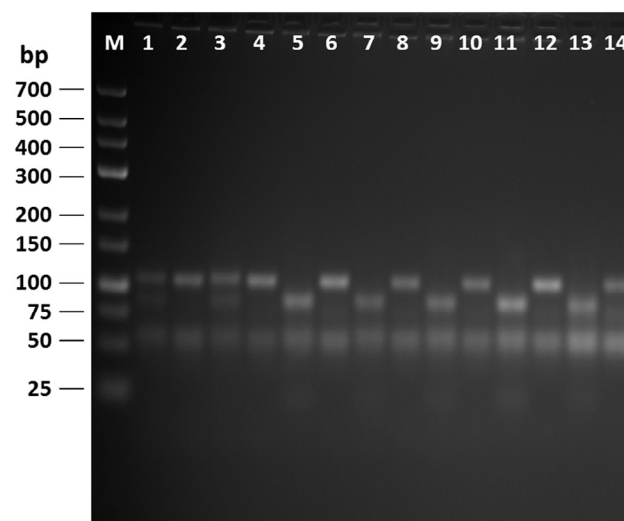


Figure 4 Agarose gel electrophoresis of PCR–RFLP fragments for *TNF-α* (−308 G/A) genotypes analysis. *NcoI* restriction site generate products of 87 bp and (20 bp not evident in gel) fragments for G allele. The A allele showed 107 bp fragment only. (GG: lanes 5, 7, 9, 11, 13 – AA: lanes 2, 4, 6, 8, 10, 12 and GA: lanes 1, 3, 14) The samples were electrophoresed against a ready-to-use, Gene Ruler™ low range DNA ladder 25–700 bp (Thermo Fisher Scientific, CA, USA), landmark bands are 100 bp and 300 bp respectively.

was topped up to 25 μl with DNase-free water. The PCR was performed on Applied Biosystems Veriti™ thermocycler (Thermo Fisher Scientific Inc., Life Technologies™, CA, USA) according to cycling conditions consisting of an initial denaturation step at 96 °C for 2.5 min, then 63 °C for 60 s

for 1 cycle, followed by 20 cycles of 96 °C for 10 s, 60 °C for 60 s for 9 cycles then 20 cycles of 96 °C for 10 s, 60 °C for 50 s, 72 °C for 30 s for, with a final extension of 72 °C for 5 min. The PCR product of 261 bp was visualized on the 2% agarose gel (see Fig. 5).

1.4.4. TGF- β gene polymorphism (+869 T/C) (rs1982073) and (+915 G/C) (rs1800471)

Genotyping was done using polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) assay as previously reported [21,22]

Amplification of a fragment spanning position (+869) was obtained using standard PCR assay, according to cycling conditions consisted of an initial denaturation step at 95 °C for 4 min, followed by 40 cycles of 95 °C for 30 s, 58 °C for 30 s and 72 °C for 30 s, with a final extension of 72 °C for 10 min. The product was digested using MspA1I (Thermo Fisher Scientific, MA, USA) for codon 10 and BglI for codon 25 (Thermo Fisher Scientific, MA, USA). The restriction digests for codon 10 (+869): T allele was recognized by fragments 40, 67, 108 and 242 bp, while the C allele showed bands of 12, 40, 67, 108, 230 bp (see Fig. 6).

1.5. Statistical analysis

Data analysis was performed using SPSS software for Windows (version 16.00, SPSS, Inc., Chicago, IL, USA). The observed alleles frequencies were compared with expected values calculated from Hardy–Weinberg equilibrium theory. The data were expressed as mean \pm SD or proportions. Chi-square test or Fisher's exact test was used for comparison of categorical variables. Student's *t*-test and ANOVA was used in the case of continuous data. All the significant variables from univariate analysis as predictors of SVR undergo stepwise multivariate logistic regression analysis. *P*-values less than 0.05 were considered to be statistically significant.

2. Results

The study included 150 consecutive chronic HCV infected adult patients (99 males and 51 females) with a mean age of

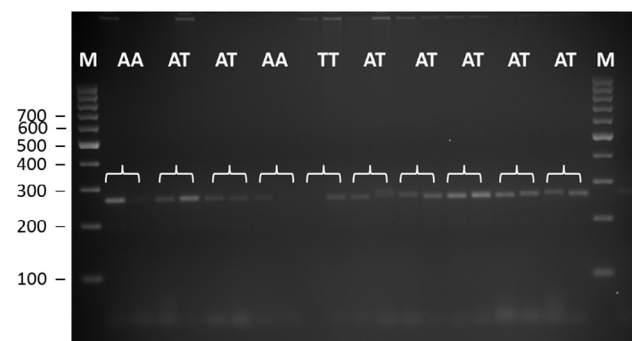


Figure 5 Agarose gel electrophoresis of primer specific PCR for IFN- γ (+874 A/T) genotypes analysis. The samples were electrophoresed against 100 bp DNA ladder (Thermo Fisher Scientific, CA, USA). PCR product of 261 bp for the two specific primers for each sample was run in two consecutive lanes and genotypes were labeled on the gel.

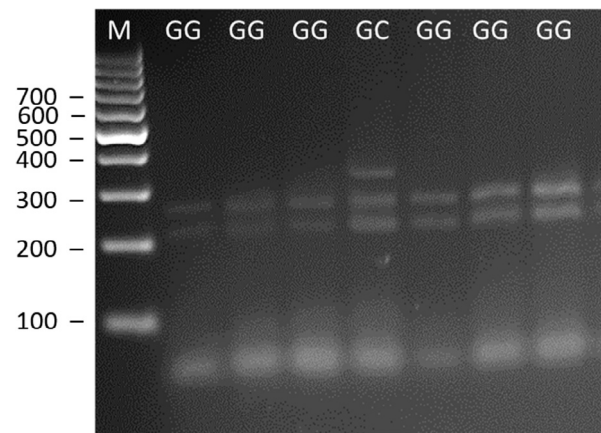


Figure 6 Agarose gel electrophoresis of PCR–RFLP for TGF- β (+915 G/C) genotypes analysis. The samples were electrophoresed against 100 bp DNA ladder (Thermo Fisher Scientific, CA, USA). Genotypes were labeled on the gel. The restriction digests for codon 25 where G allele was recognized by fragments 212, 252 (60 bp band was not evident on gel), while the C allele showed two bands of 212 and 312 bp.

41.60 \pm 9.42 years. In addition, 100 apparently healthy subjects (66 males and 34 females) with mean age 43.2 \pm 5.72 years served as the control group. Overall, 83 (55.3%) patients achieved SVR. Eighteen (12%) patients were relapsers, while 49 (32.7%) patients were non-responders. Patients who achieved SVR were younger, had lower BMI, less fibrosis and less likely to be diabetic (Table 2). There was no significant difference of various polymorphic genetic patterns in chronic HCV patients compared to controls (Table 3).

- Patients with GG genotype of IL-10 (–1082 G/A) were more likely to have SVR than patients with GA or AA genotypes (Table 4). Odds ratio (OR) was 1.98 (95% confidence interval (CI): 1.34–3.65, *p* value <0.05) for GG genotype versus other genotypes.
- There was no significant difference between various genotype patterns of IL-10 (–819 C/T) and IL-10 (–592 C/A) and also IFN- γ (+874 A/T), TNF- α (–308 G/A) and TGF- β codon 10 (+869 T/C) and TGF- β (+915 G/C) as regards SVR (Table 3).
- Multivariate analysis of the factors which were significant in the univariate analysis revealed that the presence of GG genotype of IL10 (–1082) and fibrosis stage were the only significant factors predicting SVR. HR 3.0 (95% CI: 1.78–6.12, *p* value <0.05) and 1.86 (95% CI: 1.35–3.17, *p* value <0.05) for IL10 (–1082) and fibrosis stage respectively.

3. Discussion

Chronic hepatitis C has been recognized as a progressive fibrotic liver disease [23]. However, little is known about the molecular and cellular mechanisms responsible for the virological response to PEG–IFN/RBV treatment among patients infected with HCV genotype 4 [24,25].

Table 2 Baseline demographic, biochemical, histological data of HCV infected patients and response to treatment.

Variables	All (n = 150)	SVR (n = 83)	Non-SVR (n = 67)	P-value
Age (years) (mean ± SD)	41.60 ± 9.42	38.38 ± 10.07	44.77 ± 8.24	< 0.05*
<i>Gender (n (%))</i>				
Male	99 (66%)	55 (66.3%)	44 (65.6%)	0.49
Female	51 (34%)	28 (33.7%)	23 (34.4%)	
BMI	26.1 ± 3.1	23.7 ± 2.3	27.4 ± 2.3	< 0.05*
ALT (IU/L) mean ± SD	43.86 ± 25.48	43.55 ± 26.04	44.33 ± 24.92	0.87
AST (IU/L) mean ± SD	44.46 ± 22.42	45.44 ± 23.50	43.00 ± 20.88	0.54
Hb (gm/dl)	13.16 ± 1.54	13.06 ± 1.67	13.15 ± 1.30	0.76
Platelets ($\times 10^3/\text{mm}^3$)	220.86 ± 71.34	230.54 ± 75.05	204.32 ± 61.93	0.06
AFP (ng/ml)	11.43 ± 5.80	11.85 ± 5.87	10.80 ± 5.72	0.36
<i>Viral load [n (%)]</i>				
< 600,000 IU/mL	103 (68.6%)	58 (69.8%)	45 (67.2%)	0.42
≥ 600,000 IU/mL	47 (31.3%)	25 (30.1%)	22 (32.8%)	
<i>Fibrosis stage [n (%)]</i>				
F1–F2	88 (58.6%)	53 (63.8%)	35 (52.2%)	< 0.05*
F3–F4	62 (41.4%)	30 (36.2%)	32 (47.8%)	
<i>Diabetes mellitus [n (%)]</i>				
Absent	125 (83.3%)	75 (90.36%)	52 (77.61%)	< 0.05*
Present	25 (16.7%)	8 (9.64.3%)	15 (22.39%)	

* P < 0.05 significant; SVR, sustained virological response, BMI, body mass index; ALT: alanine transaminase; AST, aspartate transaminase.

Table 3 Genotyping patterns comparison between patients and healthy controls.

	HCV patients (n = 150)		Healthy control (n = 100)		P-value
	N	%	N	%	
<i>IL-10 (-1082 G/A)</i>					
• G/G	34	22.7	17	17	0.7
• G/A	63	42.0	57	57	
• A/A	53	26.0	26	26	
<i>IL-10 (-819 C/T)</i>					
• C/C	70	46.7	44	44	0.92
• C/T	56	37.3	39	39	
• T/T	24	16.0	17	17	
<i>IL-10 (-592 C/A)</i>					
• C/C	60	40	49	49	0.2
• C/A	63	42	40	40	
• A/A	27	18	11	11	
<i>IFN-γ (+874 A/T)</i>					
• A/A	29	19.3	17	17	0.89
• A/T	85	56.7	58	58	
• T/T	36	24.0	25	25	
<i>TNFα (-308 G/A)</i>					
• G/G	96	64.0	73	73	0.32
• G/A	43	28.7	22	22	
• A/A	11	7.3	5	5	
<i>TGF-β codon 10 (+869 T/C)</i>					
• T/T	42	28	32	32	0.71
• T/C	78	52	47	47	
• C/C	30	20	21	21	
<i>TGF-β codon 25 (+915 G/C)</i>					
• G/G	127	84.7	84	84	0.95
• G/C	22	14.7	15	15	
• C/C	1	0.7	1	1	

P-value is not significant (> 0.05).

Table 4 Genotyping patterns comparison according to response to therapy.

	SVR	Relapser	Non-responder	P-value
All (N = 150)	83 (100%)	18 (100%)	49 (100%)	
<i>IL-10</i> (-1082 G/A)				0.004
GG (N = 34)	23 (27.7%)	2 (11.1%)	9 (18.3%)	
GA (N = 63)	39 (47.0%)	3 (16.7%)	21 (42.9%)	
AA (N = 53)	21 (25.3%)	13 (72.2%)	19 (38.8%)	
<i>IL-10</i> (-819 C/T)				0.74
CC (N = 70)	41 (49.4%)	6 (33.3%)	23 (46.9%)	
CT (N = 56)	28 (33.7%)	9 (50%)	19 (38.8%)	
TT (N = 24)	14 (16.9%)	3 (16.7%)	7 (14.3%)	
<i>IL-10</i> (592 C/A)				0.14
CC (N = 60)	38 (45.8%)	3 (16.7%)	19 (38.8%)	
CA (N = 63)	30 (36.1%)	12 (66.7%)	21 (42.9%)	
AA (N = 27)	15 (18.1%)	3 (16.6%)	9 (18.3%)	
<i>IFN-γ</i> (+874 A/T)				0.38
AA (N = 29)	19 (22.9%)	2 (11.1%)	8 (16.3%)	
AT (N = 85)	48 (57.8%)	9 (50%)	28 (57.2%)	
TT (N = 36)	16 (19.3%)	7 (38.9%)	13 (26.5%)	
<i>TNF-α</i> (-308 G/A)				0.33
GG (N = 96)	53 (63.9%)	15 (83.3%)	28 (57.1%)	
GA (N = 43)	24 (28.9%)	2 (11.1%)	17 (34.7%)	
AA (N = 11)	6 (7.2%)	1 (5.6%)	4 (8.2%)	
<i>TGF-β</i> (+869 T/C)				0.43
TT (N = 42)	22 (26.5%)	3 (16.6%)	17 (34.7%)	
TC (N = 78)	45 (54.2%)	9 (50%)	24 (48.9%)	
CC (N = 30)	16 (19.3%)	6 (33.4%)	8 (16.4%)	
<i>TGF-β</i> (+915 G/C)				0.35
GG (n = 127)	68 (81.9%)	14 (77.7%)	45 (91.8%)	
GC (N = 22)	14 (16.9%)	4 (22.3%)	4 (8.2%)	
CC (N = 1)	1 (1.2%)	0 (0%)	0 (0%)	

It has become clear that bi-allelic polymorphisms at positions -1082, -819 and -592 have important implications in differential IL-10 expression in humans [26]. There are conflicting data on the role of the regulatory IL-10 in HCV infection. The polymorphisms in the IL-10 gene promoter may be a host factor affecting viral persistence and response to IFN- α therapy [27].

In this study, no significant difference was observed between patients and control subjects regarding IL-10 polymorphic genetic patterns which reflect that different genetic patterns of IL-10 do not influence HCV infection susceptibility. These results are corresponding to previous reports which found that the distribution of IL-10 promoter gene polymorphisms at positions -1082, -819 and -592 was comparable between HCV patients and healthy controls [28,29].

In contrast, Vidigal et al. [30] reported that IL-10 gene promoter -1082 GG genotype occurred more frequently in HCV patients than in controls and the GG homozygosity was associated with poor response to IFN. However, this study focused on a limited number of both patients and control subjects.

The present study confirmed that the IL-10 polymorphism at position (-1082) GG genotype is a good predictor of SVR, independent of other factors, such as age, gender and viral load. The findings demonstrated in the current study are consistent with previous reports showing that PEG-IFN/RBV therapy is more effective at blocking the production of HCV in patients with 1082GG IL-10 promoter genotype and that

the rate of viral decline is increased in patients with the GG compared to the GA or AA genotypes, which correlates with higher rates of SVR [29,31,32].

Previously published data found that IL-10 genetic polymorphism has been correlated with poor response to interferon therapy [29,30,33-37]. The reasons for these discrepancies are not clear. The study populations may differ with regard to factors that influence SVR: viral load, obesity, fibrosis, ethnicity and sex. Additional studies are required to define the roles of these factors in connection with IL10 polymorphisms in chronic hepatitis C.

At the same time, we did not find any relationship between IL-10 promoter polymorphism at positions -819 and -592, and response to IFN therapy. Many authors failed to find any relationship between the IL-10 promoter polymorphism at positions -819 and -592 with the response to IFN therapy [29,30,33-37]. On the contrary, an association between the -819T and -592A SNP with SVR was reported in other studies in which -819T/T and -592A/A genotypes were significantly associated with SVR to IFN- α therapy [38,39].

Our findings could be explained on the basis that genetic polymorphism in the IL-10 gene promoter leads to alterations of specific transcription factor recognition sites. This substitution may affect transcriptional activation, leading to differing rates of IL-10 mRNA synthesis and cytokine production [40]. IL-10, produced by monocytes, macrophages and T cells, inhibits both the activation of CD4 + T-helper cells and the

function of cytotoxic CD8 + T, NK and antigen-presenting cells, and also modulates hepatic stellate cell collagen synthesis. IL-10 plays a regulatory role in immune reaction and suppresses inflammatory responses by inhibiting the production of pro-inflammatory cytokines. It may decrease circulating TNF- α and IL-6 levels, thus, reducing their harmful effects [32,41–43].

There are conflicting data about the role of IL-10 in HCV-immune response. Elevated IL-10 production is believed to mitigate the effects of IFN, which involves direct inhibition of IFN-activated signal transducer and activator of transcription-1 (STAT1) in the liver, thus inhibiting the transcription of interferon-stimulated genes, leading to the decreased expression of certain proteins with known antiviral activity [44–46].

The present study also analyzed the association between IFN- γ (+874 A/T), TNF- α (–308 G/A) and TGF- β (+869 T/C and +915 G/C) gene polymorphism and response to combined therapy in this cohort of CHC patients. No significant difference was observed between patient and control groups regarding these SNPs which reflect that these different genetic patterns do not influence HCV infection susceptibility. They also have no relation to the response to therapy. These results were in agreement with previous works conducted by Abbott et al. [47], Dai et al. [48] and Sarvari et al. [49] who did not demonstrate any association between the genotype patterns of IFN- γ (+874 A/T) with either viral clearance or treatment response. Romani et al. [22] also did not find any relation between TGF- β (+869 T/C) gene polymorphism and treatment response. As regards TNF- α , Schiemann et al. [50] reported that there was no correlation between +308 TNF- α promoter polymorphisms and the response to combination therapy of PEG-IFN/RBV in patients with chronic HCV infection.

In conclusion, we proved that HCV genotype 4 infected patients with –1082 GG genotype achieved better response to therapy. Well-adapted therapies should be based on the baseline characteristics of the patients and should follow a response-guided approach. According to our data, the presence of the 1082 GG IL-10 promoter genotype may indicate a high probability of achieving SVR.

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Conflict of interest

The authors declare that they have no conflict of interests.

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