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Interferon-Lambda 4 Gene Polymorphisms Predict Treatment Response in Egyptian HCV Genotype 4 Patients Exposed to Radiation

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ABSTRACT

Background: Direct-acting antivirals (DAAs) has seen a significant increase of the count of patients with hepatitis C virus (HCV) clearing their infection. Interferon Lambda Four (IFNL4) polymorphism plays a distinguished role in spontaneous and treatment-output clearance of HCV infection.

Aim of this Work: This study aimed to assess IFNL4 polymorphism among hepatitis C Egyptian patients who were exposed to radiation, compared to normal by a polymerase chain reaction with restriction of fragment length polymorphism (PCR-RFLP) technique.

Materials and Method: This study included 50 HCV-positive Egyptian patients working in Egyptian Nuclear materials Authority treated with DAAs therapy. According to treatment, they were split into two groups. Group I included 40 patients with sustained viral response (SVR). Group II included 10 patients with no response (nSVR). Fifty healthy people served as controls. Liver function tests, complete blood count, evaluation of viral markers, HCV-RNA by PCR, and evaluation for IFNL4 single nucleotide polymorphisms for rs368234815 were performed by PCR-RFLP in all patients. **Results:** Of the 50 patients, 40 (80%) achieved sustained virological response (SVR). Of the 23 patients with rs368234815 TT/TT genotype, 21 (91.3%) achieved SVR, while in 27 patients with non‑TT/TT genotypes, 19 (70.4%) achieved SVR. The rs368234815 was a powerful predictor of SVR. However, in the present research individuals, the predictive power of this SNP was the same as that of rs12979860 SNP.

Conclusion: In Egyptian HCV-positive patients with genotype 4, IFNL4 rs368234815 SNP is an autonomous predictor of SVR to DAAs treatment.

Keywords: Interferon Lambda Four, hepatitis C, Polymerase Chain Reaction.

INTRODUCTION

Hepatitis c virus (HCV) infects more than 170 million people globally, 70% of whom become long-term carriers. Only a minority of infected people spontaneously clear the virus, while 30 to 60% develop chronic liver illness and a significant proportion develop chronic liver disease and a substantial percentage develops cirrhosis or even hepatocellular carcinoma (HCC). Egypt has the largest hepatitis C incidence in the world [(1)]. Ionizing radiation and hcv infection communicate to boost the risk of HCC supermultiplicatively. Ionizing radiation at elevated concentrations of exposure considerably improves HCC hazards when hcv is not identified at the same time. This fits into a pattern with other studies of interaction in hepatocarcinogenesis. Synergistic or greater than multiplicative interactions in HCC being reported when subjects are subjected to agents such as radiation that cause mainly genetic alteration, as well as agents such as hcv and heavy drinking that trigger liver cell regeneration. People with HCV may therefore be particularly susceptible to exposure to radiation and vice versa [(2)].

HCV treatment was tied to combination therapy consisting of pegylated interferon and ribavirin (PEG-IFN and RBV). Recently, new antiviral agents known as direct-acting antivirals (DAAs) have been developed and introduced for treatment of HCV infection. Although DAAs are more effective than PEG-IFN/RBV combination therapy given, these new treatments of HCV are not affordable and available in many countries. PEG-IFN and RBV still known to remain the alternative HCV treatment regimen [(3)].

Egypt Demographic and Health Surveys (EDHS) assessed the incidence of antibodies among adults aged 15–59 at 14.7% in 2009 and 10.0% in 2015 substantially greater than worldwide rates. To address this challenge, Egypt created a domestic HCV control strategy and established programs for HCV prevention and therapy. Following effective 99% discounted DAA pricing negotiations, Egypt introduced an ambitious domestic HCV therapy program aimed at treating more than 250,000 chronically infected people per year with the objective of attaining a domestic chronic infection incidence of < 2% by 2025. Despite this advancement, current proof indicates that HCV transmission in Egypt continues, with greater rates of incidence compared to other nations [(4)].

Diagnostic instruments, such as genotyping IFNL3 andIFNL4, can predict those most likely to spontaneously clear HCV that could be postponed in therapy. These instruments may also stratify people and

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prioritize DAAs therapy to those less likely to react to interferon-containing regimens or recognize those likely to react well to shortened therapy [5].

Evaluations of African American patients with HCV infection indicated that IFNL4 rs368234815 may be the strongest host genetic factor for prediction of HCV treatment response. Designing a simple, inexpensive and rapid method for genotyping of the IFNL4 rs368234815 polymorphism might be helpful for predicting response to treatment [6]. The polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) method that had been developed for genotyping of rs368234815 is accurate, inexpensive, fast and simple to perform [3].

SUBJECTS AND METHODS

In this study, the cohort consisted of 100 subjects; 50 healthy control included 21 (42%) women and 29 (58%) men and their ages ranged from 18 to 57 years and 50 patients with chronic HCV infection where 14 (28%) were females and 72% were males received treatment by DAAs during the period from July 2016 to January 2018 and whose specimens were referred to the Egyptian Nuclear Material Authority Laboratory (located in Cairo, Egypt). According to the response to therapy, they were classified into two groups. The first group included patients with SVR: the chronic HCV patients who had received the therapy and had shown negative HCV-RNA after not less than 6 months of completion of a 24-week treatment course with DAAs. The second group included nonresponders to the therapy (no disappearance of HCV-RNA at the end of the 12 weeks).

Ethical Approval:

Informed consent was obtained from all patients before the study and **this study was approved by the Ethical Committee of the Nuclear Material Authority**, Cairo, Egypt. The study followed the principles of the Declaration of Helsinki.

Treatment regiments

Patients received triple combination therapy with 400 mg sof once daily, Peg-INF (180 mcg/0.5 ml; fixed dose/week) plus RBV (1000 mg for ≤75 Kg or 1200 mg for >75 Kg) for 12 weeks.

Blood sampling

All chronic HCV patients were of genotype 4 by the INNoLiPA (Fujirebio Europe, NV, Ghent, Belgium) test. Two blood samples from each topic were acquired. In serum-separating pipes, the first sample was gathered. Blood was left to clot at room temperature, then centrifuged at 4000-6000 rpm. Serum was segregated for biochemical assessment (SGOT and SGPT). The second sample was collected in a clean and dry vacutainer tube with ethylene diamine tetra acetic acid (EDTA) anticoagulant for hematology analysis (CBC) and IFNL4 rs368234815 genotypes distribution study.

DNA extraction

DNA extraction was done by using G-spin™ total DNA extraction kit according to manufacturer's instructions (Spain, Cat. No. 17045). In brief, samples were lysed and the DNA was captured on the spin columns provided. The DNA was washed, eluted and then stored at -20°C until used in PCR amplification.

IFNL4 Genotyping via PCR-RFLP

IFNL4-F primer (5'-GACGCAGGACCCCTTGGGACAGGA-3') and IFNL4-R primer (5'-TCTGGGCCGCAGTGGCCGCGAGG-3') used as a forward and reverse primer pair. The PCR reactions were performed using Accupower PCR PreMix. Amplified 100 ng to 300 ng of genomic DNA using 10 pmol of IFNL4-F and IFNL4-R primer pairs. The PCR temperature profile was as follows: 94°C for 5 minutes, 40 cycles of 94°C for 20 seconds, 60°C for 30 seconds, and 72°C for 30 seconds, followed by 72°C for 5 minutes. For the RFLP analysis, the PCR product of rs368234815 was digested with 10 units of MspA1I (New England BioLabs) restriction endonuclease (RE) for more than 1 hour. The digested PCR products were separated on 3% agarose gel alongside the GeneRuler 100-bp DNA Ladder (Thermo FisherScientific Inc.). The agarose gel was stained with the addition of 0.5 mL of DNA safe Stain (CinnaGen Co.) into each 100 mL of agarose gel. In each run of PCR-RFLP genotyping of rs368234815, as a control for enzymatic activity, a single rs368234815-ΔG/ΔG DNA specimen was included.

Statistical analysis

The data were collected, tabulated, and analyzed using statistical package for the social sciences (SPSS Inc., SPSS Statistics for Windows Version 21.0. Chicago, USA) on an IBM compatible computer. The c2-test was conducted to study the association between two qualitative variables. Fisher's exact test was used in the analysis of 2 × 2 contingency tables.

The Student t-test was used for comparison between two groups having normally distributed quantitative variables. The Mann–Whitney U-test was conducted as a nonparametric test of significance for comparison between two groups having abnormally distributed quantitative variables. Analysis of variance was used as a parametric test for comparison between more than two groups having normally distributed quantitative variables. The Kruskal–Wallis test was used as a nonparametric test of significance for compariso between more than two groups having not normally distributed quantitative variables. Regression analysis is a statistical process for estimating the relationships among variables [7].

RESULTS

Table (1): Comparison between HCV patients and controls regards to the laboratory findings.

Parameters	Mean ± SD		t-test	P Value
	Control	Patients		
TLC (10^3/µL)	4.99 ± 1.36	6.74 ± 1.89	5.31	<0.001 HS
HB (g/dL)	14.42 ± 1.92	13.63 ± 1.97	2.03	<0.05 S
PLT (10^3/cmm)	229.62 ± 39.82	220.60 ± 57.54	0.91	>0.05 NS
AST (U/L)	25.36 ± 3.76	30.68 ± 13.59	2.67	<0.05 S
ALT (U/L)	23.12 ± 3.68	30.58 ± 15.85	3.24	<0.001 HS

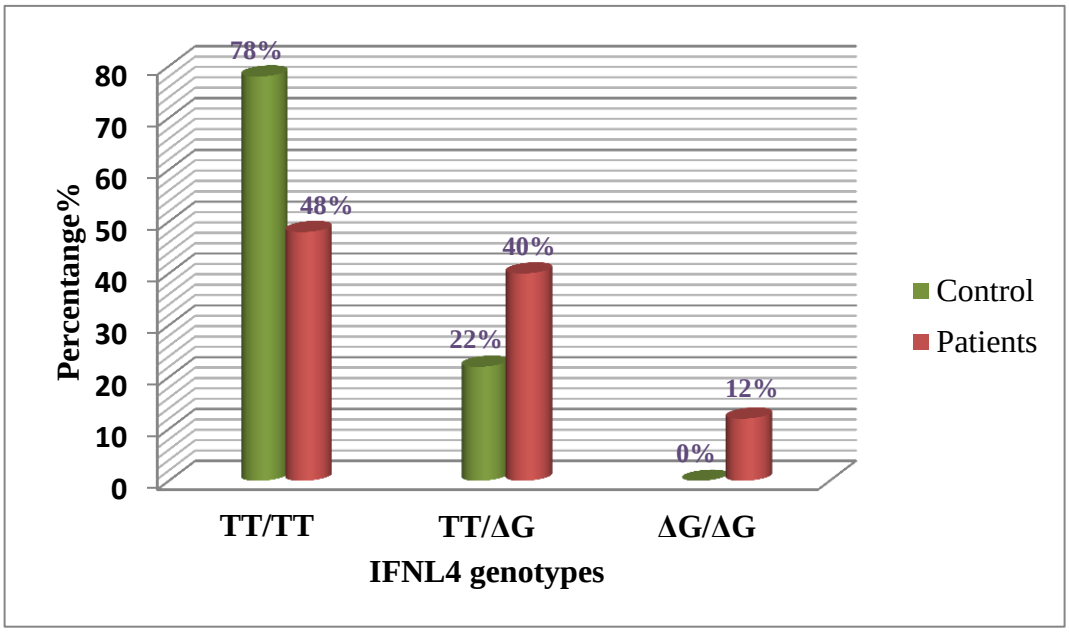

Figure (1): Distribution of the IFNL4 genotypes among hepatitis C virus (HCV) patients and healthy controls.

Table (2): Comparison between genotypes distribution and allele frequencies of IFNL4 polymorphism (rs368234815) and sustained viral response in hepatitis C virus patients (SVR and nSVR groups)

IFNL4 Polymorphism rs368234815	SVR (n = 40)	nSVR (n = 10)	OR (95 %CI)	P-value *
Genotype [n (%)]				
TT/TT	21 (52.5%)	2 (20%)	Ref (1.00)	
TT/ΔG	15 (37.5%)	5 (50%)	3.5 (0.60 – 20.52)	0.165 NS
ΔG/ΔG	4 (10%)	3 (30%)	7.88 (0.99 – 63.31)	0.052 NS
Dominant model [a]				
TT/TT	21 (52.5)	2 (20.0)	Ref (1.00)	
TT/ΔG+ΔG/ΔG	19 (47.5)	8 (80.0)	4.42 (0.83 – 23.47)	0.081 NS
Recessive model [b]				
TT/TT+TT/ΔG	36 (80.0)	7 (70.0)	Ref (1.00)	
ΔG/ΔG	4 (20.0)	3 (30.0)	3.86 (0.70 – 21.15)	0.12 NS
Alleles [n (%)]				
TT	57 (71.0)	9 (45.0)	Ref. (1.00)	-
ΔG	23 (29.0)	11 (55.0)	3.03 (1.11 – 8.28)	**0.03** S

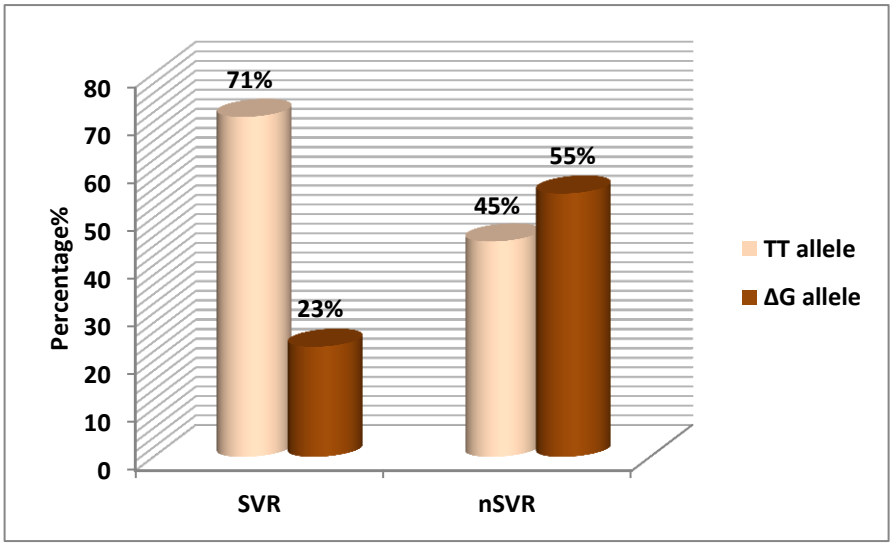

Figure (2): Alleles frequency of IFNL4 polymorphism (rs368234815) in SVR and nSVR groups.

Table (3): Comparison between IFNL4 genotypes and laboratory parameters for HCV patients

	IFNL4 genotypes			Kruskal–Wallis Test	P Value
	TT/TT (N = 23)	TT/ΔG (N = 20)	ΔG/ΔG (N = 7)		
Age (X ± SD)	50.61 ± 9.01	50.70 ± 9.98	43.71 ± 9.62	3.235	0.198 ^{NS}
TLC (X ± SD)	6.70 ± 1.68	6.69 ± 2.29	7.03 ± 1.45	0.228	0.892 ^{NS}
HB (X ± SD)	13.77 ± 2.16	13.34 ± 1.98	14.01 ± 1.32	1.209	0.546 ^{NS}
PLTs (X ± SD)	230.48 ± 55.53	210.10 ± 85.21	218.14 ± 65.26	1.122	0.571 ^{NS}
AST (X ± SD)	27.43 ± 9.97	30.25 ± 13.42	42.57 ± 19.16	4.855	0.088 ^{NS}
ALT (X ± SD)	25.87 ± 10.54	31.6 ± 16.44	43.14 ± 22.72	4.935	0.085 ^{NS}

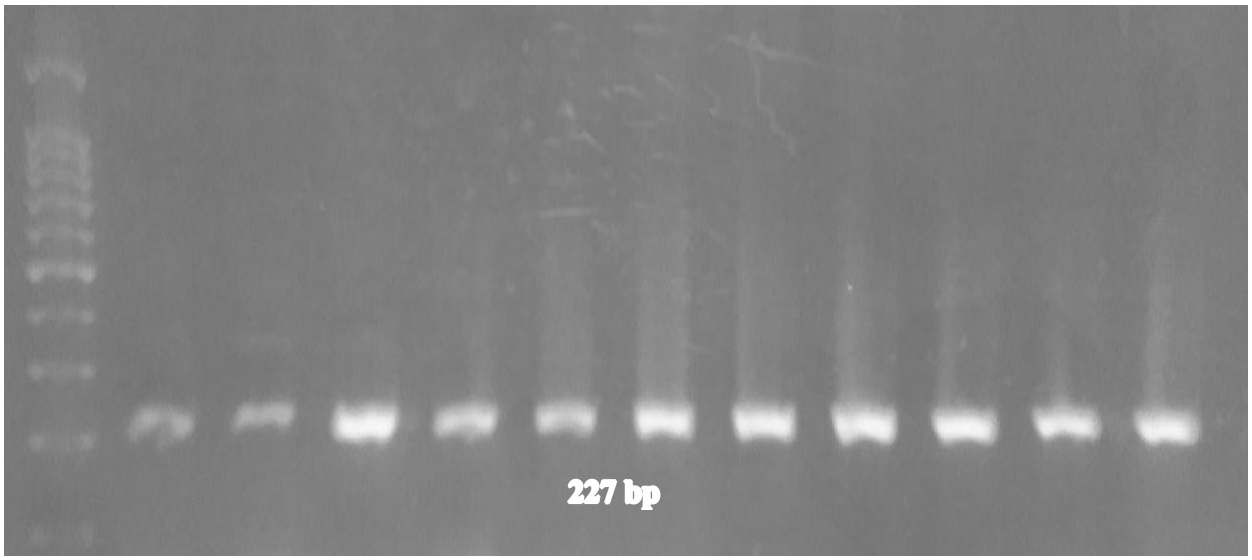

Figure (3): Agarose gel electrophoresis illustrated the PCR-RFLP products for the IFNL4 gene polymorphism. Lane 1 corresponds to 100 bp molecular weight marker, the rest are nondigested PCR product at 227 bp.

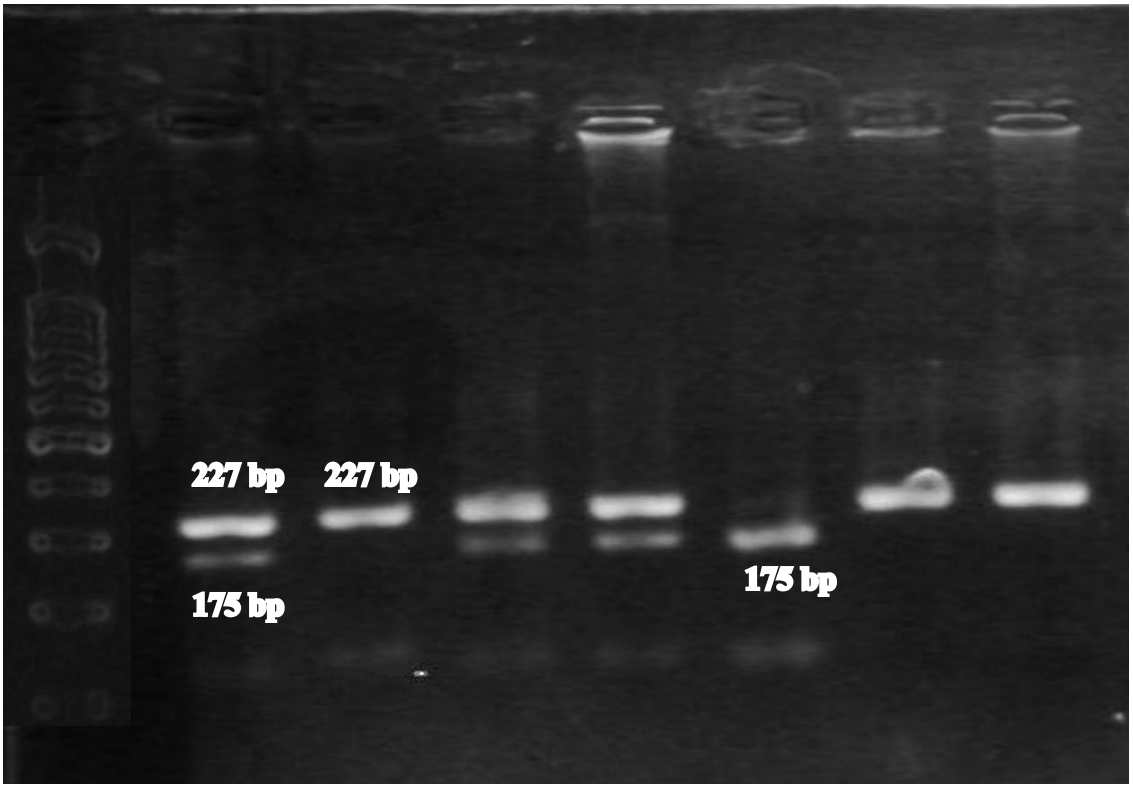

Figure (4): PCR-RFLP products on agarose gel electrophoresis illustrating for the IFNL4 gene polymorphism after difestion by MspA1I.

Lane 1 corresponds to 100 bp molecular weight marker. Lanes 3, 7, and 8 correspond to blood samples from homozygous TT/TT participants. Lanes 2,4 and 5 correspond to blood samples from homozygous TT/ΔG participants. Lane 6 correspond to blood samples from heterozygous ΔG/ ΔG participants.

DISCUSSION

The present work aimed to assess the predictive value of the IFNL4 polymorphism in the treatment of chronic HCV in Egyptian patients and its allele frequency in HCV patients compared with controls.

The mean age was 41.28 ± 8.11 and 48.18 ± 8.15 for control and patients respectively, and the difference is considered to be extremely statistically significance ($p < 0.001$).

Among the baseline laboratory values, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were significantly higher in patients than in controls ($P<0.01$). Hematology values showed that there was a significant difference as regards HB ($P<0.05$), highly significant difference as regards TLC ($P<0.01$) and no significant difference as regards PLT ($P>0.05$) between control and patients group as shown in table (1).

In This study, the 50 chronic HCV patients, the rs12979860 TT/TT, TT/ ΔG, and ΔG /ΔG genotypes were present in 48, 40, and 12% of patients, respectively, but for controls the frequency was 78, 22, and 0% for TT/TT, TT/ ΔG, and ΔG /ΔG, respectively, with no statistically significant difference between them (Fig. 1).

IFNL4-F and IFNL4-R primer pair were used for PCR-RFLP genotyping of IFNL4 rs368234815, which amplified the 226 bp/227 bp DNA fragment (Figure 3). Digestion of this product with MspA1I in individuals with the ΔG/ΔG genotype showed 2 fragments of 175 bp and 51 bp; 3 fragments of 227, 175, and 51 bp in the TT/ΔG genotype and a 227-bp fragment in the TT/TT genotype (Figure 4).The frequency of IFNL4 genotype in genotype 4 Egyptian patients working in Egyptian Nuclear Authority was: 23 TT/TT (46%), 20 TT/ΔG (40%), 7 ΔG/ΔG (14%) (Table 4). Nearly similar results were reported in many recent studies. **Pouryasin *et al***. [3] reported that the frequencies of TT/TT, TT/ΔG and band ΔG/ΔG were 44.8%, 37.9%, and 17.3%, respectively. **Galmozzi *et al***. [8] found that the genotypic frequencies of rs368234815 variant was 37% for TT/TT, 49.5% for TT/ΔG and 13.5% for ΔG/ΔG. While, **Backus *et al***. [9] showed that among 92 African Americans, 43.5% (40) had ΔG/ΔG genotype, 52.2% (48) had ΔG/TT genotype and 4.3% (4) had TT/ TT genotype. The comparison between different IFNL4

genotypes (TT/TT, TT/ΔG, and ΔG/ΔG) in HCV patients where there was no significant difference as regards Age, TLC, HB, PLTs, AST and ALT (Table 3). In this study, the distribution pattern of IFNL4 genotypes among HCV patients as regards response to treatment was 40 (80%) of the studied patients achieved an SVR, whereas 10 (20%) did not. Of the 40 patients with an SVR, 21 had genotype TT/TT, 15 had genotype TT/ΔG, and four had genotype ΔG/ΔG with no statistically singnificant difference (P>0.05). However, there was a significant difference between SVR and nSVR as regards the IFNL4 alleles, as the frequency of the TT allele was higher in SVR than in nSVR (71% vs. 45%) and the ΔG allele was lower in HCV patients than in controls (29% vs. 55%) (Table 6).

Prokunina-Olsson *et al*. [(10)] showed that the polymorphism rs368234815 (ΔG) resulted in a frame shift mutation and therefore produced a new gene designated as interferon-lambda 4 (IFNL4). Homozygous individuals with IFNL4 TT genotype could not create this gene. Researchers have indicated that IFNL4 ΔG genotype is correlated with poorer treatment reaction relative to TT genotype.

CONCLUSION

The IFNL4 polymorphism is an independent predictor of SVR to DAAS in Egyptian HCV patients with genotype 4. Besides, increasing the chances of achieving SVR, determining IFNL4 SNPs before initiating treatment will be cost-effective and will reduce adverse effects.

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