

Relevance of Interferon Lambda 4 and Endoplasmic Reticulum Amino Peptidase Polymorphism to HCV Response to Treatment

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ABSTRACT

Objective: The present study aims to evaluate the relevance of IFN λ 4rs73555604 SNP and ERAP1rs26618 SNPs and response to direct anti-viral drugs among HCV infected Egyptian patients.

Participants and Methods: 100 participants were included in the study (40 HCV infected patients responded to DAA therapy, 40 HCV infected patients who did not respond to DAA and 20 healthy volunteers). Allelic discriminations of IFN λ -4 rs73555604SNP and ERAPrs26618SNP were carried out using step One real-time PCR.

Results: Allelic discrimination of IFN λ 4rs73555604 and ERAP1rs26618 exhibited significant differences among the studied groups where 75% of non-responders carry IFN λ 4rs73555604T allele while 75% of responders carry ERAPrs26618C allele. Calculations of odds ratio revealed that persons carrying T allele of IFN λ 4rs73555604 and ERAPrs26618 have 2.5 and 3.3 folds more odd to be non-responder to DAA therapy, respectively.

Conclusion: IFN λ -4rs73555604 and ERAP-1rs26618 polymorphism could affect HCV response to DAA treatment among HCV infected Egyptian patients. There was a significant association between IFN λ 4rs73555604CC genotype and response to treatment. However, ERAP1rs26618T-allele was associated with resistance to treatment. Odds ratio calculation revealed that persons carrying ERAPrs26618T allele have 3.3 folds more odd to be non-responder to DAA therapy. Both SNP can be used as surrogate markers to assess response to DAA treatment.

KEY WORDS

hepatitis C virus, IFN λ 4rs73555604 SNP, ERAP1rs26618 SNP, viral response to direct antiviral agents

BACKGROUND

Hepatitis C virus (HCV) is a positive sense ssRNA virus that has seven genotypes^{1,2)}. It frequently establishes chronic infection leading to chronic hepatitis, hepatocellular carcinoma (HCC), hepatic failure, and death. HCC is considered the second cause of cancer deaths all over the world³⁾.

In Egypt, nearly 15% of the population are suffering from chronic hepatitis C (CHC), due to infection by HCV genotype 4. This results in a great social and economic burden. In the past, interferon (IFN- α) with ribavirin was used in treatment of HCV. December 2013, Sofosbuvir in combination with Ribavirin was approved by FDA for the treatment of

HCV genotypes 4²⁾.

HCV eradication, either by interferon-alfa (IFN- α) or direct antiviral agents (DAA), is influenced by a complex interaction between host genetic, environmental, and viral factors. Many genome-wide associated studies (GWAS) have been performed to clarify the effects of human gene polymorphisms on HCV clearance among different populations^{3,4)}.

Interferon-lambda-4 (IFN λ 4) belongs to type III interferons (IFN-III). It has an important role against hepatitis C viral (HCV) infection and can affect viral response to treatment by controlling expression of the antiviral IFN-stimulated gene (ISG)⁵⁾. IFN λ 4 is a functional gene in > 95% of the African population and is located upstream of IFNL3⁶⁾. It was suggested that polymorphism of IFN λ 4 gene may affect its expression and so can predict response to treatment⁷⁾. IFN λ 4 ss469415590 gene

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Table 1: Comparison between the three studied groups according to hematological findings

Hematological investigations	HCV infected patients		Negative control (n = 20)	Test of sig.	p
	Responders (n = 40)	Non-responders (n = 40)			
Hemoglobin (g/dl)				F = 12.203*	< 0.001*
Mean ± SD.	12.59 ± 1.63	13.84 ± 1.74	14.51 ± 0.63		
Sig. bet. gps	p ₁ = 0.001*, p ₂ < 0.001*, p ₃ = 0.262				
RBCs (x10⁶/cmm)				F = 5.176*	0.007*
Mean ± SD.	4.65 ± 0.62	4.99 ± 0.75	5.25 ± 0.85		
Sig. bet. gps	p ₁ = 0.085, p ₂ = 0.008*, p ₃ = 0.379				
WBCs (x10³/cmm)				H = 10.963*	0.004*
Mean ± SD.	5.54 ± 2.09	6.80 ± 2.09	6.88 ± 1.53		
Sig. bet. gps	p ₁ = 0.006*, p ₂ = 0.005*, p ₃ = .566				
Platelets (x10³/cmm)				F = 5.094*	0.008*
Mean ± SD.	158.0 ± 24.95	140.30 ± 44.91	169.75 ± 44.15		
Sig. bet. gps	p ₁ = 0.006*, p ₂ = 0.556, p ₃ = 0.268				

F: F for ANOVA test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Tukey)

H: H for Kruskal Wallis test, Pairwise comparison bet. each 2 groups was done using Post Hoc test.

p: p value for comparing between the studied groups, p1: p value for comparing between responders and non-responders

p2: p value for comparing between responders and negative control, p3: p value for comparing between non-responders and negative control, *: Statistically significant at p ≤ 0.05

Table 2: Comparison between the three studied groups according to biochemical investigation

Biochemical investigations	HCV infected patients		Negative control (n = 20)	Test of sig.	p
	Responders (n = 40)	Non-responders (n = 40)			
AST (SGOT) (U/L)				H = 43.227*	< 0.001*
Mean ± SD.	46.45 ± 25.04	66.83 ± 30.10	24.70 ± 5.03		
Sig. bet. gps	p ₁ < 0.001*, p ₂ < 0.001*, p ₃ < 0.001*				
ALT (SGPT) (U/L)				H = 39.966*	< 0.001*
Mean ± SD.	41.58 ± 20.47	60.48 ± 20.96	24.60 ± 5.12		
Sig. bet. gps	p ₁ < 0.001*, p ₂ = 0.001*, p ₃ < 0.001*				
Albumin (g/dl)				F = 18.568*	< 0.001*
Mean ± SD.	4.28 ± 0.44	4.01 ± 0.52	4.78 ± 0.39		
Sig. bet. gps	p ₁ = 0.025*, p ₂ < 0.001*, p ₃ < 0.001*				
TSB (mg/dl)				H = 46.662*	< 0.001*
Mean ± SD.	0.80 ± 0.17	0.86 ± 0.18	0.32 ± 0.16		
Sig. bet. gps	p ₁ = 0.247, p ₂ < 0.001*, p ₃ < 0.001				
AFP (ng/ml)				H = 64.814*	< 0.001*
Mean ± SD.	7.88 ± 4.99	18.70 ± 10.99	0.53 ± 0.63		
Sig. bet. gps	p ₁ < 0.001*, p ₂ < 0.001*, p ₃ < 0.001*				
PT (seconds)				H = 50.316*	< 0.001*
Mean ± SD.	82.63 ± 7.56	86.28 ± 5.52	12.05 ± 0.76		
Sig. bet. gps	p ₁ = 0.110, p ₂ < 0.001*, p ₃ < 0.001*				
INR value				F = 47.066*	< 0.001*
Mean ± SD.	1.19 ± 0.20	1.20 ± 0.08	0.86 ± 0.05		
Sig. bet. gps	p ₁ = 0.992, p ₂ < 0.001*, p ₃ < 0.001*				

F: F for ANOVA test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Tukey), *: Statistically significant; H: H for Kruskal Wallis test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test.

p: p value for comparing between the studied groups, p1: p value for comparing between responders and non-responders

p2: p value for comparing between responders and negative control, p3: p value for comparing between non-responders and negative control, *: Statistically significant at p ≤ 0.05.

otypes were found to have a predictive value on HCV response to pegylated interferon-α and ribavirin (PEG IFN-α/RBV) therapy in the Chinese population⁹.

Additionally, machinery of antigen-processing consists of the proteasome (site of degradation of exogenous antigens), transporters for antigen presentation that translocate peptide precursors, endoplasmic reticulum aminopeptidases (ERAPs), including ERAP1 and ERAP2, which trim the peptides to fit major histocompatibility complex (MHC), and MHC proteins that present antigen peptides on the cell surface⁹.

Recently, several studies have shown that ERAP proteins play crucial roles in infectious diseases⁹, autoimmune diseases¹⁰, and cancers^{8,11}. ERAP1 gene polymorphisms result in changes in its aminopeptidase activity which in turns may influence the activation of immune response and/or response to treatment¹¹.

Thus the aim of the present study is to appraise the relevance of IFNλ4rs73555604 and ERAP1rs26618 single nucleotide polymorphisms (SNPs) to the response to direct anti-viral agents among HCV infected Egyptian patients.

Table 3: Allele frequency and odd ratio for IFN λ 4rs555604 and ERAPrs26618 among the studied groups

	Negative control (n = 20)		Responders (n = 40)		Non-responders (n = 40)		OR	95% CI		p
	No.	%	No.	%	No.	%		LL	UL	
IFNλ4rs555604										
CC®	4	20.0	13	32.5	2	5.0	-	-	-	-
TC	14	70.0	11	27.5	16	40.0	9.454*	1.771	50.47	0.009*
TT	2	10.0	16	40.0	22	55.0	8.937*	1.765	45.25	0.008*
Allele										
C®	22	55.0	37	46.3	20	25.0	-	-	-	-
T	18	45.0	43	53.8	60	75.0	2.581*	1.321	5.046	0.006*
ERAPrs26618										
CC®	12	60.0	20	50.0	6	15.0	-	-	-	-
TC	6	30.0	20	50.0	26	65.0	4.333*	1.467	12.79	0.008*
TT	2	10.0	0	0.0	8	20.0	-	-	-	-
Allele										
C®	24	60.0	60	75.0	38	47.5	-	-	-	-
T	16	40.0	20	25.0	42	52.5	3.316*	1.697	6.479	0.001*

OR: Odds ratio (comparison between responders and non-responders); CI: Confidence interval, LL: Lower limit, UL: Upper Limit, *: Statistically significant at $p \leq 0.05$

PARTICIPANTS AND METHODS

Study design

The study included 100 participants divided into 3 groups; **group 1:** 40 HCV infected patients responded to DAA therapy, **group 2:** 40 HCV infected patients who did not respond to DAA therapy and **group 3:** 20 healthy volunteers matched in age and sex with HCV infected patients. DAA Treatment regimen was daily sofosbuvir (400 mg) + daily daclatasvir (60mg) \pm ribavirin (1000 mg if weight 75 kg or 1200 mg if \geq 75 kg) for 12 weeks. Patients under 20 and over 56 years old, co-infected with hepatitis B virus (HBV) or suffering from HCC were excluded from the study.

Ethical considerations

The study protocol and procedures were approved by the ethics committee of Faculty of Medicine, Kafrelsheikh University Egypt (2-016) 2017 ; and informed written consents were gathered from all participants. The study conforms to ethical guidelines of the 1975 Declaration of Helsinki.

Data and Sample Collection

Full history taking, and thorough clinical examination were performed for all individuals under the study. Seven ml of venous blood samples were collected; two ml in ethylene diamine tetra-acetic acid (EDTA) tubes for complete blood count (CBC), three ml in plain test tubes for serum separation (to be used in biochemical and viral investigations), and two ml in EDTA tubes for DNA extraction and molecular assay.

Hematological and biochemical investigations:

Complete blood count (CBC), prothrombin time, liver functions' tests, international normalized ratio (INR), and alfa-fetoprotein (AFP) were assessed for all participants⁽¹²⁻¹⁴⁾.

HCV-RNA "viral load" by Real time PCR

Active viral replication was determined by measuring HCV-RNA levels using real time polymerase chain reaction (RT-PCR) after three months of completion of therapy to evaluate response to therapy⁽¹⁴⁾.

Detection of IFN λ -4 rs7355604 and ERAP rs26618 polymorphisms

DNA was isolated and purified from whole blood (EDTA) using QIAamp - spin columns (QIAamp DNA Blood Mini Kit, Applied Biosystems-Life Technologies); according to the manufacturer instructions⁽¹⁵⁾. Allelic discriminations of IFN λ -4 rs7355604 and ERAP rs26618 SNP were carried out using step One RT-PCR (Applied Biosystem-Life Technologies, Carlsbad, California, USA). Each TagMan® SNP genotyping assay contains sequence-specific forward and reverse primers, two allele-specific TagMan® minor groove binder (MGB) probes for distinguishing between the two alleles; VIC® fluorescent dye is linked to the 5' end of C allele's probe and FAM (6-carboxyfluorescein) fluorescent dye is linked to the 5' end of T allele 's probe and non-fluorescent quencher (NFQ) at the 3' end of each probe⁽¹⁶⁾. All reagents were brought to room temperature (20-25°C) prior to use. In PCR tubes, 12.5 μ l TagMan Universal PCR master mix, 1.25 μ l TagMan SNP Genotyping Assay, 9.25 μ l DNase-free water and 2 μ l genomic DNA were mixed together to reach reaction volume of 25 μ l. The thermal cycling conditions were programmed as follow: 95°C for 10 min., 40 cycles of 92°C for 15 sec and 60°C for 1 min., then hold at 4°C. A significant increase in FAM dye only indicates homozygosity for the wild allele (C/C), VIC dye only means homozygosity for the mutant allele (T/T) and if both fluorescence signals increase, this indicates heterozygosity (C/T)⁽¹⁷⁾.

Statistical analysis of the data

Data were analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). Qualitative data were described using number and percent. The Kolmogorov-Smirnov test was used to verify the normality of distribution. The studied sample population was explored to find its equilibrium with Hardy-Weinberg equation (HWE). Quantitative data were described using minimum, maximum, mean, and standard deviation. Significance of the obtained results was judged at 5% level^(18,19).

RESULTS

Hardy-Weinberg equation (HWE) was done to check for any possible deviations within all participants regarding the studied genotypes. No deviation was observed ($P > 0.05$).

Participants demographic data:

The mean ages among responders, non-responders and control

groups were 48.70 ± 7.8 , 49.20 ± 8.95 and 46.15 ± 14.85 years old, respectively. It was ranging between 32 - 66 years, 35 - 65 years and 19 - 61 years, respectively. There were no statistically significant differences between ages of the studied groups ($p = 0.523$).

Regarding sex distribution, the male to female ratios among responders, non-responders and control groups were 6.5: 3.5 (26 males:14 females), 6.7: 3.2 (27 males: 13 females) and 1: 1 (10 males, 10 females), respectively. There was no statistical difference among HCV infected patients and controls ($p = 0.393$).

Hematological investigations:

Hematological investigations were done for all participants and results illustrated in **Table (1)**. Hb levels were significantly reduced among responders when compared with non-responders ($p = 0.001^*$) and control group ($P < 0.001^*$). Red blood cells¹ (RBCs) counts were markedly decreased among responders in comparison to control group ($p = 0.008^*$). Measures of white blood cells (WBCs) count revealed statistically significant leucopenia among responders in comparison to non-responders ($p = 0.006^*$) and control ($p = 0.005^*$) groups. Platelets counts were markedly increased among responders when compared with non-responders ($P = 0.006^*$).

Biochemical investigations

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, total serum bilirubin (TSB), PT, AFP, and INR were determined for all participants (**Table 2**). Statistical analysis revealed a significant increase in AST, ALT, and AFP serum levels among non-responders when compared with responders and control groups. Also, their levels were high among responders when compared to control groups. HCV infected patients (responders and non-responders) have decreased serum albumin and increased TSB, PT and INR when compared with control group ($P < 0.001^*$).

HCV Viral load

HCV viral load was determined for all participants. All responders were below detection limit (< 15 IU/ml), however, it ranged between 0.12×10^6 and 60.98×10^6 IU/ml with the mean value of $5.57 \times 10^6 \pm 11.85 \times 10^6$ IU/ml among non-responders.

Genotypic distribution of IFN- λ -4 rs73555604 SNP and ERAP rs26618SNP:

IFN λ 4rs555604 and ERAP rs26618 genotyping was based on 5' nuclease allelic discrimination assay (TaqMan assay). There was a significant difference among the studied groups where ERAPrs26618 T allele is highly represented among non-responders while ERAPrs26618 C allele is more common among responders and controls ($P = 0.001^*$). There was a significant association between IFN λ 4rs555604 CC genotype and response to treatment ($P = 0.004^*$). Correlation analysis did not show any effect of the demographic and laboratory data on the response to treatment. Calculations of odds ratio and confidence interval showed no significant difference in genotypic distribution of IFN- λ -4 rs73555604 SNP and ERAPrs26618 SNP between HCV infected patients and negative controls. However, odds ratio calculation for responders vs non-responders revealed that persons carrying T allele of IFN λ 4rs555604 and/or ERAPrs26618 have 2.5 and 3.3 folds, respectively, more odd to be non-responder to DAA therapy, respectively (**Table 3**).

DISCUSSION

Hepatitis C virus is the commonest blood-borne virus for which a vaccine is still lacking. It is estimated that about 3% of the world's population, approximately 200 million individuals, are infected with HCV²⁰. In Egypt, nearly 15 million are suffering from HCV infection with about 200,000 new cases and 40,000 deaths each year. This high prevalence was due to reuse of needles during mass-treatment programs for schistosomiasis and till now transmission continues to occur via contaminated injections, and dental care²¹.

HCV is considered the main cause of liver cirrhosis, fibrosis and HCC. So great efforts have been done to develop treatments for HCV

starting from interferon and ribavirin combination therapy to the new direct acting antiviral therapies that provide significantly higher cure rates and good tolerability profiles²¹.

Many genome-wide association studies (GWASs) have identified host genes associated with the different outcomes of HCV infection in different world populations^{2-4,11,16,22-24}. IFN λ 4 is a new type III IFN and its expression is controlled by some SNP within or near IFN λ 4 gene as rs368234815, rs12979860 and rs8099917. It was reported that IFN λ 4 genotype may affect the intrahepatic expression levels of IFN-stimulated genes (ISGs) that affect the responsiveness to IFN- α ⁶. Additionally, abnormalities of ERAP1 could result in alteration in HLA-I expression and consequently impaired T-cell responses. This in turn may affect susceptibility to infection, activation of immunity and/or response to treatment as SNPs located in the ERAP1 gene result in changes of the amino acid sequence, and affect ERAP1 enzymatic properties¹¹.

The current study intended to appraise the relevance of IFN λ 4 rs73555604 and ERAP1 rs26618 to the response of HCV infected Egyptian patients to treatment with DAA. All patients were treated with sofosbuvir (Sovaldi, 400mg, daily) + daclatsvir (60 mg, daily) \pm ribavirin for 12 weeks. Regarding demographic data, results revealed that the majority of patients were in the fifth and sixth decades of life and this correlates well with the results obtained by other studies^{25,26}.

Regarding CBC, there was a significant reduction of Hb level, RBCs and platelets count among HCV patients ($p = 0.001^*$). This may be explained by low nutritional status of the patients during the course of disease and/ or as a side effect of long term combination treatment of sovaldi and ribavirin that may cause hemolytic anemia. In accordance to our results, **Nemr N., et al (2016)²⁷** and **Kamilia G., et al., (2019)²⁸** have reported that Ribavirin induces thrombo-cytopenia among Egyptian patients with hepatitis C. Moreover, **Zanaga LP., et al., (2019)²⁹** has reported that the most common side effect of direct antiviral drugs is anemia.

ALT and AST elevation indicates hepatocyte injury. Very high ALT and AST is linked to CHC progression, high risk for cirrhosis, and resistance to treatment³⁰. In the current study, there was marked increase in AST and ALT serum levels among non-responder group when compared to both responder and control group ($p = 0.001^*$). Decrease of ALT and AST among responders' group reflects the effectiveness of the treatment used by decreasing hepatic inflammation and necrosis²⁹. Regarding serum bilirubin, there was a significant increase in serum bilirubin among non-responder group in comparison with both responder and control group ($P = 0.001^*$). Additionally, responders showed slightly raised levels when compared to control group. This could be attributed to the inhibition of the bilirubin transporters and possibly ribavirin induced hemolysis.

As regards HCV viral load, we found that HCV decline and clearance occurred after initiating the DAA treatment among most of patients. This was consistent with normalization of elevated ALT and AST levels. In agreement with our results; **Attar BM and Van-Thiel DH. (2016)³¹** have demonstrated the benefit of DAA treatment not only for HCV RNA clearance but also for normalization of ALT and AST.

IFN λ 4 rs73555604 (C17Y) is a common nonsynonymous SNPs in exon 1, with high frequency in African ancestry (26%)⁷. It is located within the leader peptide and thus might be functionally important for modulation of IFN λ 4 activity⁷.

Results in the current study clarified that a significant association between IFN λ 4rs555604 CC genotype and response to treatment ($P = 0.004^*$). However, correlation analysis did not show any effect of the demographic and laboratory data on the response to treatment. **Terczyńska-Dyla, E., et al., (2014)³²** have stated that genetic variation within IFN λ 4 locus affects both spontaneous and treatment-induced clearance of HCV. Changing a proline to a serine at position 70 of IFN λ 4 protein results in alteration of its antiviral activity as patients harbouring the impaired IFN λ 4-S70 variant display lower interferon-stimulated gene (ISG) expression levels, and better treatment response rates, compared with patients coding for the fully active IFN λ 4-P70 variant. **Sakhae F, et al., (2017)²⁹** have reported that IFN λ 4 rs469415590 can predict SVR among HCV infected Iranian patients. **Ramamurthy N., et al., (2018)³³** have reported that HCV genotype 3 infected patients carrying IFN λ 4 rs12979860 CC genotype had 2-folds higher likelihood to achieve SVR compared to TT genotype. **Pedergnana V, et al., (2019)³³** have explored the relation between IFN λ 4 genotypes and response to IFN and/or DAA regimens. They noticed that IFN λ 4 rs368234815 SNP was associated with DAA-based SVR among patients infected with HCV genotype 3 form BOSON and United Kingdom. In the current study, we estimated the effect size (odds ratio; OR) of IFN λ 4 between cases (responders and non-responders) and control groups. No devia-

tions from HWE were observed within all participants ($P > .05$). The allelic distribution of rs73555604 among cases and controls was significantly different, and the T-allele was associated with resistance to treatment compared with the C-allele ($P = .006$, OR = 2.581*, 95%CI: 1.321- 5.046). Also both TC and TT genotypes was significantly different between all participants in comparison CC genotypes.

On the other hand, Saafan AE, *et al.*, (2020)²¹ have compared the antiviral efficacy of dual and triple Sovaldi combination therapy among 100 HCV positive patients and estimated SNP genotyping of INFL-4 gene by real time PCR. They reported that SNP in IFN λ 4 gene has no effect on response to Sofosbuvir in Egypt either in dual therapy or triple therapy.

Regarding allelic and genotypic frequencies of ERAP1 polymorphisms, several variants have been linked to human diseases; Yao Y. *et al.*, (2016)¹¹ have compared frequencies of ERAP1 SNPs, rs26653GC, rs26618TC, rs30187CT, and rs27044CG, among non-small cell lung carcinoma (NSCLC) patients in Chinese Han and Polish Caucasians. They noticed marked associations of all four SNPs with NSCLC in Chinese but not in Poles (except for rs26618). They attributed this to the difference in these genotype frequencies between Chinese and Poles. Another possible explanation lies in disparities of HLA alleles frequencies between Orientals and Caucasians³⁴. Some peptides presented by MHC-I are dependent on ERAP1 trimming while other peptides are independent on ERAP1 and can fit MHC molecule without trimming³⁵.

In the current study, it was noticed that ERAP1 rs26618 T-allele was associated with resistance to treatment when compared with the C-allele ($P = 0.001$, OR = 3.316*, 95% CI: 1.697-6.479). TC genotype was significantly different between responders and non-responders in comparison to both CC and TT genotypes. This could be attributed to the fact that change or loss of ERAP1 could result in a marked disturbance in antigen-processing pathway and shift in the hierarchy of immune dominance during viral infections. This is because ERAP1 trims precursor peptides in the endoplasmic reticulum to generate or destroy antigenic peptides. Consequently, changes in ERAP1 could result in reduction or increase of the immune response to viral peptides. Moreover, ERAP1 rs26618 leads to an amino acid substitution (I276M)⁹. This in turn could affect the viral response to treatment.

On contrary to our result, Liu S, *et al.*, (2017)³⁶ have investigated the association between HCV chronic infection and ERAP gene SNPs (rs27044, rs30187, rs26618 and rs26653) in ERAP1 and 2 SNPs (rs2248374 and rs2549782) in ERAP2 genes in Chinese Han HCV chronic infections and healthy controls. They found that ERAP1 rs26618 C-allele is associated with increased risk of HCV chronicity in comparison with the T-allele ($P = .025$, OR = 1.318, 95%CI: 1.035-1.677). There were notable differences in the genotype distribution in analysis using the dominant genetic model in ERAP1 rs26618 SNP (CC + CT vs. TT; $P = 0.007$, OR = 1.473, 95%CI: 1.091-1.989). They concluded that ERAP-1 rs26618 SNP may play a critical role in HCV chronicity in Chinese Han population. Guasp P., *et al.* (2016) have stated that ERAP-1 rs26618 SNP (I276M) may affect the peptidome of ERAP1 by destroying peptides with p2 Ala, unless the p1 amino acid was resistant to ERAP1 trimming³⁷. This in turn could affect its activity in antigen processing and presentation.

The main limitation of the study is the small sample size. Thus further researchers are recommended to verify the effect of these polymorphisms among HCV infected patients receiving direct antiviral drugs.

CONCLUSION

IFN- λ -4 rs73555604 and ERAP-1 rs26618 polymorphism could affect HCV response to DAA treatment among HCV infected Egyptian patients. There was a significant association between IFN λ 4rs555604 CC genotype and response to treatment. However, ERAP1 rs26618 T-allele was associated with resistance to treatment. Odds ratio calculation revealed that persons carrying T allele of ERAPrs26618 have 3.3 folds more odd to be non-responder to DAA therapy. Both SNP can be used as surrogate markers to assess response with different DAA regimens used in treatment of HCV.

CONFLICT OF INTERESTS

Authors declare that there is no conflict of interests or any financial

supports regarding the publication of this research.

ETHICS STATEMENT

The study protocol and procedures were approved by the ethics committee of Faculty of Medicine, Kafrelsheikh University Egypt (2-016) 2017 ; and informed written consents were gathered from all participants. The study conforms to ethical guidelines of the 1975 Declaration of Helsinki.

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