Type of the Paper (Article)

The effect of the polymorphism in IL-28B gene on the treatment response of a combined therapy by sofosbuvir and daclatasvir with and without ribavirin in HCV Egyptian patients

Eman E. Mahmoud[1], Hoiyda A. Abdel Rasoul[1], Eriny H. I. Abdallah[1*], Essam A. Hassan[2]

[1] Department of Clinical and Chemical Pathology, Faculty of Medicine, Fayoum University, Fayoum, Egypt.

[2] Department of Tropical medicine Faculty of Medicine, Fayoum University, Fayoum, Egypt.

Abstract

Introduction: HCV infection is a universal health condition. Polymorphisms at the interleukin 28B (IL-28B) gene severely affect treatment results to direct-acting antiviral drugs (DAA), including Sofosbuvir and Daclatasvir with or without Ribavirin. The relationship between the virus and the host immune system is critical for revealing the prognosis and the response to medications.

Aim of our study: It aimed to reveal if there is an association between the SNP of the IL-28B (rs12979860) gene and hepatitis C virus treatment response to DAA in an Egyptian population.

Subjects and methods: This case-control descriptive analytical thesis contains 200 subjects, 100 HCV patients who received Sofosbtuvir, Dacatasvir ± Ribavirin (50responders&50 non-responders) compared to 100 age and sex-matched healthy control subjects. Diseased people were recruited from the Tropical clinic in Fayoum University Hospital from May 2019 to February 2020. All samples were subjected to genomic DNA analysis of SNP of the IL-28B gene (rs12979860) using Real-Time PCR.

Results: The SNP IL-28B gene (rs12979860) was not statistically significantly different between HCV-diseased people compared to healthy subjects ($P > 0.05$). The c allele was also higher among drug-responsive patients, while the T allele was higher among drug-resistant patients ($P < 0.01$), which is a highly statistically significant difference.

Conclusion: The study showed a significant relation between IL-28Bgene (rs12979860) polymorphism and hepatitis C virus treatment response to DAA. CC genotype is a significant indicator of SVR in HCV genotype 4 Egyptian patients, and this genotype may influence the effectiveness of treatment.

Keywords: IL-28Bgene; polymorphism; sustained virological response.

* Correspondence: Eriny H. I. Abdallah, ehi01@fayoum.edu.eg; Tel.: (002) 01091425455.

1. Introduction

The Hepatitis C virus (HCV) infection is a universal health condition. Nearly 71 million people have chronic hepatitis C virus (CHC) infection worldwide. Egypt has been detected as the country with the highest spread of HCV by

the World Health Organization (WHO) [1]. A Survey regarding Egyptian Health Issues (EHIS), in 2015, revealed that 10% of Egyptian people from the age 15 to 59 were infected by HCV, and 7% of them developed a chronic form of active hepatitis [2]. HCV is a major leading factor of hepatic cirrhosis, hepatic carcinoma, and liver transplantation universally. Diseased people with HCV need potent medications to avoid developing these morbidities and then decrease mortalities [3]. Oral direct-acting antiviral (DAA) medications are highly potent and safe in their efficacy [4]. Direct-acting antiviral medications were categorized by their mechanism into three major categories: Non-Structural protein 3/4, A protease inhibitors, and NS5B polymerase inhibitors (e.g., sofosbuvir) [5]. Many DAA combination medications taken orally for the treatment of HCV were studied in many types of research, which 17 revealed increased Sustained Virological Response (SVR) rates with few side effects [6]. The prognosis of hepatitis C virus infection and the response to medications are determined by viral factors and human immunity [7]. Gene polymorphisms detected in cytokines, e.g., interferon- λ (IFNλ), reveal possible relationships among the viral genotypes and the prognosis of HCV-associated hepatic disease. Interleukin -28B (IL-28B) is a cytokine gene that encodes interferon gamma three protein, which is why the IL-28 gene is named interferon gamma 3 [8]. Cytokines stimulate human inflammatory reactions as a major mechanism of human defense in response to viral infections [9]. Decreased production of cytokines in hepatitis C virus infections could cause chronic infection or be unresponsive to medications [10]. Single nucleotide polymorphism (SNPs) in the IL-28B gene has been identified to be associated with HCV treatment response. In 2009, a genome-wide association study (GWAS) revealed that SNP at IL-28B (rs12979860) is the strongest host genetic factor of sustained viral response in HCV genotype1. Between the three genotypes of IL-28B, CC genotypes are accompanied by a 2:3 times increase in SVR in comparison with CT or TT genotypes [11].

2. Subjects and methods

2.1. Subjects

The current case-control descriptive-analytical study included 200 subjects: 100 subjects with HCV compared to 100 healthy control subjects.

Inclusion criteria

- Group 1: HCV RNA positivity, age 18-75 years.
- Group 2: Age and sex-matched healthy control subjects.

Exclusion criteria

Patients with either total serum bilirubin > 3 mg /dl, Serum albumin < 2.8 g/dl, INR > 1.7, and platelet count < 50000/mm were excluded. All cases of the Hepatocellular carcinoma, except for the duration of six months after surgery for cure

aim with no activity indicated by CT or MRI, were excluded as well. Patients with Extra-hepatic malignancy, except after two years free of malignancy (thier treatment might start soon after the cure from lymphoma and chronic lymphocytic leukemia, depending on the oncology report), pregnant female patients (or not using protective contraception), or patients with uncontrolled diabetes mellitus (HbA1c > 9) were excluded as well.

2.2. Study design

All cases were subjected to the following:

1. Detailed history, including age and sex.
2. Blood chemistry including ALT, AST, total bilirubin, and albumin (all were done on Beckman colter Au 680 automated chemistry analyzer).
3. Full blood examination, including TLC, Hb, and platelets (all were done on Sysmex XN 1000).
4. Coagulation profile (Was done by STA COMPACT).
5. HCV RNA titration in HCV cases.
6. Presence or absence of diabetes.
7. FIB4: Fibrosis-4 score helps to estimate the amount of scarring in the liver, as follows:

Age (years) × AST Level (U/L) =

Platelet Count (10^9/L) × ALT (U/L)

Data was retrieved from the files of the patients.

8. Clinical Examination, including anthropometric measurements of height, weight, and BMI.

Molecular tests

Genomic DNA analysis of IL-28B polymorphism (rs12979860) by Real-time PCR.

2.3. Statistical analysis:

Statistical analyses were done using the SPSS (statistical package for social science, Chicago, USA) version 17.

Data were subjected to the Kolmogorov–Smirnov test to show the distribution and method of analysis. Skewed data are shown as median (range), and statistical significance was tested using the Mann–Whitney test. The variation in categories is shown in percentages. The quantitative variables with normal distribution are shown like mean ±, and comparing groups were done by student's t-test. The Chi-square test was used to compare the demographic data (sex), genotype, and allele distributions, clinical data (drug-responsive and medication non-response) among the cases, and the controls using odds ratios and a 95 % confidence interval. Allele frequencies were determined by the gene counting method and showed as allele total number percentage. All frequencies of genotypes were subjected to Hardy–Weinberg analysis in 2 groups by using the $\chi 2$ test. When *P-value* (two-tailed) is below 0.05, it is considered statistically significant.

3. Results

3.1. Relationship among different IL-28B genotypes and SVR in hepatitis C virus patients

Table 1 and Figure 1 and 2 reveals that among the 50 responder and 50 non-responder HCV patients, there were 22 (44 %) and 10 (20 %) with the CC genotype, respectively (CC is considered our homozygous wild reference genotype). Additionally, the genotype TT of IL-28B (rs12979860) percentage is much higher in non-responders (22%) when compared to the responders (6%), which is highly statistically significant (*P<0.01*). The elevation of frequency of the TT genotype in non-responder patients reveals that the TT genotype could have the responsibility for the non-response to DCV and SOF medications in HCV patients. CT genotype has statistical significance in responders in comparison with non-responders (*P<0.05*).C allele percentage is more in responders compared to non-responders, while the T allele percentage is more in non-responders in comparison with responders, which is highly statistically significant (*P<0.01*).

Table 1: Relationship among different interleukin 28B genotypes and sustained viral response in HCV patients.

Variable		**Responder (N= 50)**	**NR (N=50)**	**Statistics**	***P-value***
Genotype	**CC N (%)**	22 (44 %)	10 (20%)	Reference	Reference
	CT N (%)	25 (50 %)	29 (58 %)	Odds ratio 2.552 95% CI 1.018-6.398	< 0.05*
	TT N (%)	3 (6%)	11 (22%)	Odds ratio 8.067 95% CI 1.837-35.413	< 0.01*
Alleles	**C (N/%)**	69 (69%)	49 (49%)	Odds ratio 2.317 95% CI 1.301-4.127	< 0.01*
	T (N/%)	31 (31%)	51 (51%)		

* Significant *P-value*.

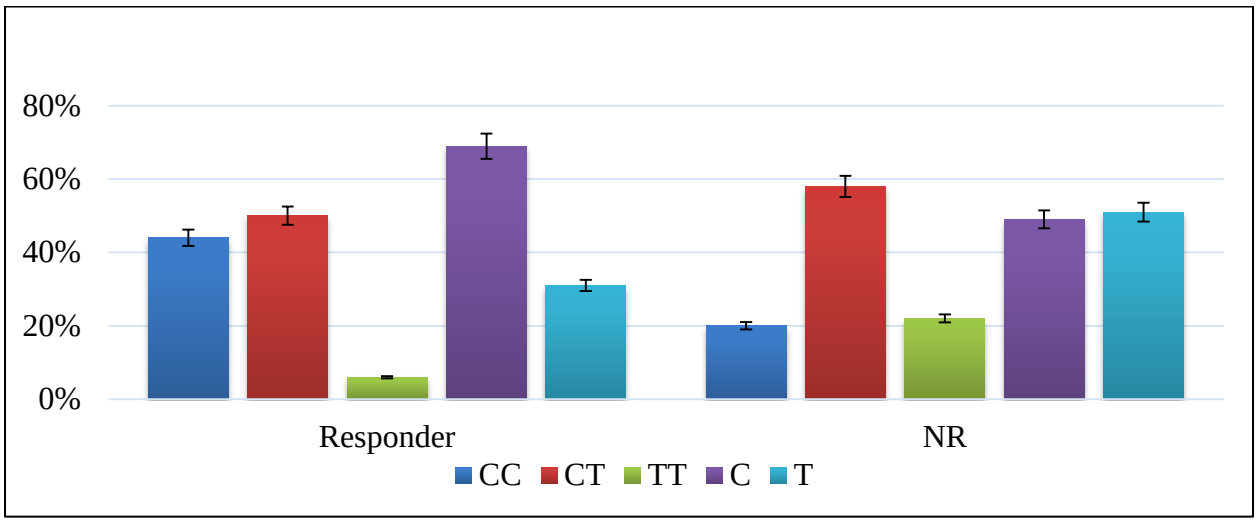

Figure 1: Genotypes and Alleles in Responder and NR.

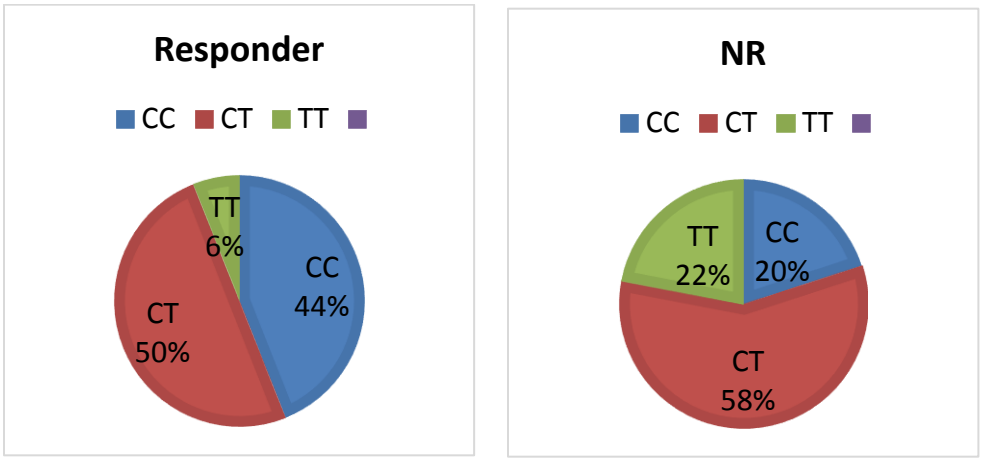

Figure 2: Genotypes in Responder and NR.

4. Discussion

In the current study, healthy control subjects were compared to each responder and non-responders: the CC genotype represents 25%, 44% & 20% in controls, responders, and non-responders, respectively. In comparison with Gomaa *et al.*, 2015, in the Egyptian population, which CC genotype represents 43.3% of controls and 91.3 % & 08.7% of responders and non-responders, respectively [12].

As regards TT genotype, it represents 18% among healthy control subjects, while

it is lower in responders 3% and higher in non-responders 22%. That was opposite to the genotype consequences of Gomaa *et al.,* 2015, study where both responders and responders had equal percentages of 50% for both, while the percentage was 1% in healthy control subjects [12]. While in the current study, CT genotype is 57% in controls, 58% in non-responders, and 50% in responders. Gomaa *et al.,* 2015, study revealed that the CT genotype was 53.3% in controls, 56.7% in responders, and 43.1 % in non-responders [12].

Regarding the T allele, it had the highest percentage in non-responders, 51%, the lowest percentage in responders, 31%, and 46.5 % in healthy control subjects. In contrast, in Gomaa *et al.,* 2015, a study in Egyptian patients T allele was highest in controls at 60 %, while at 54 and 46 in responders and non-responders, respectively [12].

In the current study, the C allele represents the highest value in responders, 69%, and the lowest value in non-responders, 31%, while in controls, 53.5%. That is in contrast to the Gomaa *et al.,* 2015, study, which revealed the highest percentage of non-responders, 44%, and the lowest of responders, 36%, while 40% of controls [12]. The higher TT genotype frequency in non-responder patients revealed that the TT genotype might have a role in non-response to combined DCV and SOF in hepatitis C virus patients.

The study conducted by Abd EL-Raheem, *et al.,* 2017, suggested that the CC genotype is a great indicator of SVR in chronic hepatitis C genotype 4 Egyptian patients [13]. However, da Silva Conda *et al.,* 2014, study did not prove a major relation between SNP rs1297960 with SVR [14].

Conclusions

The data of this study revealed that the TT (minor) genotype of IL-28B (rs12979860) could affect the result of combined DCV and SOF therapy± Ribavirin. The rise in TT genotype percentage among non-people who do not respond to treatment indicates that TT genotype may have a role in non-response to combined DCV and SOF for preserving the SVR in chronic HCV patients. Also, the study showed that the CC (major) genotype and C allele of IL-28B (rs12979860) was found in more frequency in SVR patients (44%) in comparison with non-responders (20 %), showing the relation of CC (major) genotype with the likelihood of inducing sustained viral response.

Our study reveals a statistically insignificant difference in IL-28B (rs12979860) genotype and allele frequency when HCV subjects when comparing HCV subjects to healthy control subjects.

Ethical Approval Statement: The study was approved by the Institutional Ethics Committee of Fayoum Faculty of Medicine, Fayoum, Egypt.

Informed Consent Statement: Written informed consent was obtained from all patients.

Funding: This research is not funded.

Conflicts of Interest: All authors declare no conflict of interest.

References

1. WHO. Hepatitis C fact sheet no. 164. 2016. https://www.who.int/news-room/fact-sheets/detail/hepatitis-c.
2. Kandeel A, Genedy M, El-Refai S, Funk AL, Fontanet A, Talaat M. The prevalence of hepatitis C virus infection in Egypt 2015: implications for future policy on prevention and treatment. Liver Int. 2017;37(1):45-53. doi: 10.1111/liv.13186.
3. Hajarizadeh B, Grebely J, Dore GJ. Epidemiology and natural history of HCV infection. Nat Rev Gastroenterol Hepatol. 2013;10(9):553-562. doi: 10.1038/nrgastro.2013.107.
4. Pol S, Corouge M, Vallet-Pichard A. Daclatasvir-sofosbuvir combination therapy with or without ribavirin for hepatitis C virus infection: from the clinical trials to real life. Hepat Med. 2016;8:21-26. doi: 10.2147/HMER.S62014.
5. Sulkowski MS, Vargas HE, Di Bisceglie AM, Kuo A, Reddy KR, Lim JK, Morelli G, Darling JM, Feld JJ, Brown RS, Frazier LM, Stewart TG, Fried MW, Nelson DR, Jacobson IM; HCV-TARGET Study Group. Effectiveness of Simeprevir Plus Sofosbuvir, With or Without Ribavirin, in Real-World Patients With HCV Genotype 1 Infection. Gastroenterology. 2016;150(2):419-429. doi: 10.1053/j.gastro.2015.10.013.
6. Kohli A, Kapoor R, Sims Z, Nelson A, Sidharthan S, Lam B, Silk R, Kotb C, Gross C, Teferi G, Sugarman K, Pang PS, Osinusi A, Polis MA, Rustgi V, Masur H, Kottilil S. Ledipasvir and sofosbuvir for hepatitis C genotype 4: a proof-of-concept, single-centre, open-label phase 2a cohort study. Lancet Infect Dis. 2015;15(9):1049-1054. doi: 10.1016/S1473-3099(15)00157-7.
7. Rehermann B. Interaction between the hepatitis C virus and the immune system. Semin Liver Dis. 2000;20(2):127-141. doi: 10.1055/s-2000-9946.
8. Sarrazin C, Susser S, Doehring A, Lange CM, Müller T, Schlecker C, Herrmann E, Lötsch J, Berg T. Importance of IL28B gene polymorphisms in hepatitis C virus genotype 2 and 3 infected patients. J Hepatol. 2011;54(3):415-421. doi: 10.1016/j.jhep.2010.07.041.
9. Thomas E, Gonzalez VD, Li Q, Modi AA, Chen W, Noureddin M, Rotman Y, Liang TJ. HCV infection induces a unique hepatic innate immune response associated with robust production of type III interferons. Gastroenterology. 2012;142(4):978-988. doi: 10.1053/j.gastro.2011.12.055.
10. Bochud PY, Bibert S, Kutalik Z, Patin E, Guergnon J, Nalpas B, Goossens N, Kuske L, Müllhaupt B, Gerlach T, Heim MH, Moradpour D, Cerny A, Malinverni R, Regenass S, Dollenmaier G, Hirsch H, Martinetti G, Gorgiewski M, Bourlière M, Poynard T, Theodorou I, Abel L, Pol S, Dufour JF, Negro F; Swiss Hepatitis C Cohort Study Group; ANRS HC EP 26 Genoscan Study Group. IL28B alleles associated with poor hepatitis C virus (HCV)

clearance protect against inflammation and fibrosis in patients infected with non-1 HCV genotypes. Hepatology. 2012;55(2):384-394. doi: 10.1002/hep.24678.

11. Thomas DL, Thio CL, Martin MP, Qi Y, Ge D, O'Huigin C, Kidd J, Kidd K, Khakoo SI, Alexander G, Goedert JJ, Kirk GD, Donfield SM, Rosen HR, Tobler LH, Busch MP, McHutchison JG, Goldstein DB, Carrington M. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. Nature. 2009;461(7265):798-801. doi: 10.1038/nature08463.
12. Gomaa SH, Abdel Mohsen MA, Mostafa HM, Ahmed MA. Interleukin 28B rs12979860 polymorphism and High serum Gamma-glutamyl transpeptidase activity Predict Non - Virological response to Interferonalpha/Ribavirin Combined Therapy in Chronic hepatitis C genotype 4 Egyptian patients. Int J Curr Microbiol App Sci. 2015; 4(4): 306-320.
13. Abd EL- Raheem HD, Medhat H, Hashem MH, Hemeida AA, Ebeed ME, Arafa UA, Yousef LM. Interleukin- 28B polymorphism is a pharmacogenetic predictor during sofosbuvir plus pegylated interferon and ribavirin therapy for chronic hepatitis C Egyptian patients. Journal of Pharmacogenomics & Pharmacoproteomics. 2017; 8:1. doi: 10.4172/2153-0645.10001.
14. da Silva Conde SR, Soares Monteiro JC, Silva Dos Santos BT, Fonseca Filgueiras NK, de Almeida Lins PA, Bonfim Freitas F, da Silva Graça E, Demachki S, Ferreira de Araújo MT, Ishak R, Rosário Vallinoto AC. SNP rs8099917 in gene IL28B might be associated with risk of chronic infection by HCV but not with response to treatment. Biomed Res Int. 2014;2014:748606. doi: 10.1155/2014/748606.