Chemokine receptor CXCR6 gene polymorphism and treatment response of chronic hepatitis C virus in Egyptian patients

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Abstract

Aim of the study: Despite achieving a high cure rate of chronic hepatitis C nowadays, treatment failure remains a major concern and host genetic polymorphism could have a possible relation. The aim was to evaluate the role of chemokine receptor CXCR6 gene polymorphism in treatment response to direct acting antivirals (DAAs) in chronic hepatitis C virus (HCV) patients.

Material and methods: We investigated the chemokine receptor CXCR6 gene single nucleotide polymorphism rs2234358 in three groups. Responder and non-responder groups (each comprising 50 naïve patients) and a control group of 50 apparently healthy individuals were studied.

Results: Genotype distribution revealed a significant difference ($p = 0.037$) between non-responders and the other 2 groups. Both control and responder groups showed allelic frequencies of 20% having the wild allele G and 80% having the variant allele T, while in the non-responder group 39% had G and 61% had the T alleles. Genotype GG was associated with significant increased risk of not responding to treatment by 4.25 times as compared with TT genotype ($p = 0.019$) and the G allele was associated with highly significant risk of not responding to treatment by 2.56 times compared with the T allele ($p = 0.003$).

Conclusions: CXCR6 gene (rs2234358) polymorphism could have a potential role in the virological treatment response with a protective effect of the T allele. This could explain the higher treatment success rate of Egyptian HCV patients.

Key words: hepatitis C virus (HCV), direct-acting antivirals (DAAs), CXCR6, polymorphism, sustained virological response.

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Introduction

Hepatitis C virus (HCV) in Egypt is a real health problem with prevalence of around 10%; in most cases it is genotype 4 [1, 2]. Before 2011, pegylated interferon with ribavirin (peg-IFN/RBV) was the only available treatment regimen, with a success rate of only 50% [3], but direct acting antivirals (DAAs) are effective at a higher rate for hepatitis C treatment [4]. They increased the success rate to reach more than 95% [1, 5]. In spite of this, these drugs are extremely costly in many countries and treatment failure means a large number of patients owing to the sheer size of the HCV-infected population [6]. The World Health Organization reported that 71 million people have chronic HCV infection [7]. Thus, it seems prudent to continue exploration of factors that might affect treatment response. Host immunity is an example; it was...
hypothesized to help DAAs to clear HCV if an immune modulating therapy is used to induce an endogenous T cell response [8] that was based on its success in spontaneous clearance of the virus in 25% of acute HCV cases [9], and the long term use of ribavirin as an immune modulator in HCV treatment. The chemokine receptor CXCR6 and its ligand CXCL16 control the migration and function of natural killer T cells and lead to CD4+ and CD8+ T cell activation, which could have an important role in the treatment response of HCV infection [10, 11]. Chen et al. in 2017 studied the CXCR6 gene variant rs2234358 and concluded that it could affect the spontaneous outcome of HCV infection and also the response to IFN/RBV treatment [12]. To our knowledge, there are no reported studies on the CXCR6 gene single nucleotide polymorphism (SNP) rs2234358 in the Egyptian populations. In fact, there are only a few published data regarding this SNP profile in African populations. Our aim was to study this SNP in the Egyptian population and to investigate the possible association between it and the response of our HCV patients treated with DAAs.

**Material and methods**

Our retrospective study included all consecutive non-responder adult (> 18 years old) naïve chronic HCV patients who received standard doses of the combination treatment 400 mg sofosbuvir and 60 mg daclatasvir (Sovaldi/Daklinza) for 3 months and recruited from the National Liver Institute outpatient clinics, Menoufia University, between January 2017 and May 2019 (non-responder group). Non-responding was defined as any detectable HCV RNA level at the end of treatment using a quantitative PCR technique with a detection limit of 15 IU/ml. Also, we included 50 naïve patients with chronic HCV infection (age and sex matched) who responded to the same treatment (Sovaldi/Daklinza) for 3 months. Responding was defined as undetectable HCV RNA at the end of treatment (responder group).

Our patients had no co-infection with HBV or HIV. They had no other causes of liver disease such as metabolic liver diseases, autoimmune liver diseases, alcoholic liver diseases and fatty liver disease. Also, patients who had advanced or uncontrolled co-morbid conditions or who received previous HCV treatment were excluded. Symptomatic (decompensated) cirrhotic patients were excluded. Patients were diagnosed as having liver cirrhosis when transient elastography was more than 16.3 kPa [13].

A third control group of 50 apparently healthy individuals (age and sex matched), with free clinical, laboratory and abdominal ultrasonography examination, were included in the study. Our study was approved by the ethical committee of the National Liver Institute, University of Menoufia. Informed written consent was obtained from all participants. The study protocol conforms to ethical guidelines of the 1975 Declaration of Helsinki as reflected in a prior approval by the institution's human research committee.

All individuals were subjected to laboratory testing of HCV-RNA levels by reverse transcriptase polymerase chain reaction (RT-PCR), liver function tests (aspartate aminotransferase [AST], alanine aminotransferase [ALT], alkaline phosphatase [ALP], γ-glutamyl transpeptidase [GGT], total, direct bilirubin, total protein, albumin, prothrombin time and international normalized ratio [INR]), serum creatinine, blood glucose level and serum α-fetoprotein beside complete blood cell count.

DNA extraction from whole blood samples of all participants was performed using an Invitrogen DNA Blood Mini Kit (PureLink 96 Genomic DNA Kit [K1821-04]). Polymorphism of CXCR6 (rs2234358) gene single nucleotide polymorphism (SNP) genotyping was done using real-time polymerase chain reaction (PCR, ABI TaqMan allelic discrimination kit). The real-time PCR fluorescence detection on an Rotor-Gene Q Real Time PCR System (Rotor-Gene Q MDX, Germany) using fluorescent labeled probes. TaqMan probes are sequence-specific oligonucleotides with a fluorophore and a quencher. TaqMan minor groove binder (MGB) probes additionally harbor a MGB moiety, which helps to increase allelic discrimination using two probes that only differ by one nucleotide. During PCR, the PCR primers anneal to the target sequence during the PCR annealing step. Two TaqMan probes, each specific for one of the available alleles (G or T), are present in the reaction, specifically annealing to the target region between the two primers. The proximity of the fluorophore with the quencher results in efficient quenching of fluorescence from the fluorophore. The Taq DNA polymerase extends the primer. When the enzyme reaches the TaqMan probe, its 5’ – 3’ exonuclease activity degrades the probe, resulting in physical separation of the fluorophore from the quencher. Increased fluorescence associated with the measured Rn values from the released fluorophore indicates which alleles are present in the sample (details in Supplement 1).

**Statistical analysis**

Data were statistically analyzed using SPSS, version 20, for Windows, and a P value was considered statistically significant if less than 0.05. Descriptive
statistics include estimates for summarizing the data as mean (X), standard deviation (SD), median (Med), and range or interquartile range (IQR) for quantitative data, and frequency with percentage (%) for qualitative data. The independent samples t-test or chi-square ($\chi^2$) test was used to examine the difference between the two groups for continuously distributed or categorical variables, respectively. Fisher’s exact test was used instead of the $\chi^2$ test when the assumption that at least 80% of the expected frequencies are greater than five was violated. Univariable and multivariable logistic regression analysis was used for identifying parameters independently associated with DAA resistance, and a $p$-value $\leq 0.1$ was set as a criterion for variable inclusion in the multivariable model.

Results

There were fifty HCV naïve patients who failed to respond to treatment (non-responder group) out of 1515 treated patients at the same time (success rate was 96.7%). Demographic and basal laboratory investigations of the three groups are summarized in Table 1. Although some liver tests (AST, ALT, ALP, GGT, total bilirubin, and total protein), beside white blood cell count, platelet number and serum creatinine level

Table 1. Demographic and basal laboratory values of the studied groups

| Parameters | Control (n = 50) | Responder (n = 50) | Non-responder (n = 50) | P value | Pairwise |
|------------|-----------------|-------------------|-----------------------|---------|----------|
| Age (years) | 49.3 ± 4.4 (41-59) | 50.3 ± 4.5 (42-59) | 51.1 ± 4.5 (43-60) | 0.134 | – |
| Sex (M/F) | 23/27 | 23/27 | 24/26 | 0.828 | – |
| Hemoglobin (g/dl) | 13.5 ± 1.3 (11.3-16.2) | 13.2 ± 1.5 (10.5-16.4) | 13 ± 1.5 (9.5-16.5) | 0.349 | – |
| WBCs ($10^3$ cells/ml) | 6.2 ± 1.1 (4.1-8.9) | 6.8 ± 2.3 (3.5-10.3) | 7.3 ± 2.1 (3.5-11.4) | 0.025 | $p_1 = 0.871$ $p_2 = 0.021$ $p_3 = 0.302$ |
| Platelets ($10^3$ cells/ml) | 278.5 ± 57 (184-420) | 218.1 ± 71.1 (119-395) | 210.9 ± 68.6 (115-455) | < 0.001 | $p_1 < 0.001$ $p_2 < 0.001$ $p_3 = 0.849$ |
| ALT (U/l) | 18.8 ± 3.7 (11-30) | 68.3 ± 41.2 (25-192) | 71.3 ± 37.4 (31-184) | < 0.001 | $p_1 < 0.001$ $p_2 < 0.001$ $p_3 = 1.000$ |
| AST (U/l) | 17.4 ± 5.7 (8-33) | 49.9 ± 30 (16-142) | 51.8 ± 26.8 (18-164) | < 0.001 | $p_1 < 0.001$ $p_2 < 0.001$ $p_3 = 1.000$ |
| ALP (U/l) | 62.4 ± 10.4 (41-80) | 64.5 ± 13.4 (42-91) | 69.5 ± 14.3 (44-95) | 0.027 | $p_1 = 1.000$ $p_2 = 0.029$ $p_3 = 0.165$ |
| GGT (U/l) | 23.6 ± 6.6 (13-40) | 45.5 ± 10.7 (21-64) | 47 ± 11.9 (21-65) | < 0.001 | $p_1 < 0.001$ $p_2 < 0.001$ $p_3 = 1.000$ |
| Total bilirubin (mg/dl) | 0.5 ± 0.2 (0.2-0.9) | 0.9 ± 0.3 (0.5-1.8) | 1.1 ± 0.4 (0.5-2.1) | < 0.001 | $p_1 < 0.001$ $p_2 < 0.001$ $p_3 = 0.06$ |
| Albumin (g/dl) | 4.4 ± 0.4 (3.7-5.1) | 4.3 ± 0.4 (3.4-5.1) | 4.2 ± 0.5 (3.4-5.2) | 0.256 | – |
| Total protein (g/dl) | 7.4 ± 0.5 (6.5-8.2) | 7.2 ± 0.5 (6.5-8.3) | 7.1 ± 0.5 (6.5-8.5) | 0.03 | $p_1 = 0.167$ $p_2 = 0.02$ $p_3 = 0.410$ |
| INR value | 1.01 ± 0.1 (0.9-1.1) | 1.04 ± 0.1 (0.9-1.2) | 1.03 ± 0.1 (0.9-1.2) | 0.26 | – |
| Creatinine (mg/dl) | 0.81 ± 0.1 (0.6-1.07) | 0.82 ± 0.2 (0.5-1.32) | 0.9 ± 0.2 (0.6-1.3) | 0.02 | $p_1 = 0.808$ $p_2 = 0.008$ $p_3 = 0.03$ |
| AFP (ng/ml) | 3.7 ± 2.1 (0.6-9.8) | 3.9 ± 2.7 (0.7-11) | 4.3 ± 2.1 (0.8-10) | 0.155 | – |
| HCV RNA level ($\times 10^6$) | – | 3.4 ± 1.8 (0.7-8.2) | 3.7 ± 2.0 (0.6-9) | 0.456 | – |

WBCs – white blood cells, AST – aspartate aminotransferase, ALT – alanine aminotransferase, ALP – alkaline phosphatase, GGT – $\gamma$-glutamyl transpeptidase, INR – international normalized ratio, AFP – $\alpha$-fetoprotein
showed statistically significant differences ($p < 0.05$) between the three studied groups, none of them showed a statistically significant relation between responder and non-responder groups, including the basal HCV viral load.

The $\chi^2$ test was used for Hardy-Weinberg equilibrium. We found that genotype distribution of the CXCR6 (rs2234358) SNP was consistent with Hardy-Weinberg equilibrium in the control group ($p = 0.458$) while it was inconsistent in both non-responder and responder groups ($p = 0.013$ and $0.044$, respectively).

In Table 2, the genotype distribution of CXCR6 SNP (rs2234358) in control, responder, and non-responder groups is shown. The genotype distribution among the three studied groups revealed a significant difference ($p = 0.037$). On adding the genotypes GG to GT in the dominant model of the wild G allele (GG + GT), the distribution between the studied groups revealed a more significant difference ($p = 0.025$). Similar results were found in the recessive model (GG), where the distribution between the studied groups also revealed a significant difference ($p = 0.027$). The allelic

| Table 2. Genotype distribution and allelic frequencies in the three groups |
|-------------------------------------------------|
| Polymorphism of CXCR6 (rs2234358)                |
| Control ($n = 50$)                              |
| Responder ($n = 50$)                            |
| Non-responder ($n = 50$)                        |
| $\chi^2$ test                                  |
| $P$-value                                      |
| Genotypes, n (%)                               |
| TT 33 (66.0)                                    |
| GT 14 (28.0)                                    |
| GG 3 (6.0)                                      |
| $\chi^2 = 10.21$                                |
| $0.037$                                        |
| Dominant model, n (%)                          |
| TT 33 (66.0)                                    |
| GG + GT 17 (34.0)                               |
| $\chi^2 = 7.35$                                |
| $0.025$                                        |
| Recessive model, n (%)                         |
| GT + TT 47 (94.0)                               |
| GG 3 (6.0)                                      |
| $\chi^2 = 7.20$                                |
| $0.027$                                        |
| Alleles, n (%)                                 |
| T 80 (80.0)                                     |
| G 20 (20.0)                                     |
| $\chi^2 = 12.41$                               |
| $0.002$                                        |

$%$ – percent of genotype or allele within group

| Table 3. Genotype distribution and allelic frequencies between responder and non-responder groups |
|-------------------------------------------------|
| Polymorphism of CXCR6 (rs2234358)               |
| Responder ($n = 50$)                            |
| Non-responder ($n = 50$)                        |
| OR (95% CI)                                     |
| $P$-value                                       |
| Genotypes, n (%)                               |
| TT 34 (68.0)                                    |
| GT 12 (24.0)                                    |
| GG 4 (8.0)                                      |
| OR = 2.19 (0.88-5.46)                          |
| $0.090^*$                                       |
| Dominant model, n (%)                          |
| TT 34 (68.0)                                    |
| GG + GT 16 (32.0)                               |
| OR = 2.71 (1.20-6.11)                          |
| $0.016^*$                                       |
| Recessive model, n (%)                         |
| GT + TT 46 (92.0)                               |
| GG 4 (8.0)                                      |
| OR = 3.24 (0.96-11.00)                         |
| $0.050^*$                                       |
| Alleles, n (%)                                 |
| T 80 (80.0)                                     |
| G 20 (20.0)                                     |
| OR = 2.56 (1.36-4.82)                          |
| $0.003^*$                                       |

$^*$ Pearson chi-square test, % – percent of genotype or allele within group
frequencies were 20% with the wild allele G and the remaining 80% had the variant allele T in both control and responder groups, while in the non-responder group there were 39% with G and 61% with T alleles.

In Table 3, the comparison of genotype distribution and allele frequencies of CXCR6 SNP (rs2234358) between responder and non-responder groups shows that GG genotype was associated with a significantly increased risk of not responding to DAAs as compared with TT genotype (p = 0.019) while GT genotype was not significantly associated with the risk of not responding as compared with TT genotype. The subjects with the wild G allele had a highly significant risk of not responding compared with variant allele T (p = 0.003). The G allele also showed a significant association with not responding to DAA therapy in the dominant model (p = 0.016), while in the recessive model, the association narrowly missed statistical significance (p = 0.050).

Table 4 shows that the distribution of CXCR6 SNP (rs2234358) genotypes, alleles, dominant, or recessive models were non-significantly different between the responding and the control groups (p > 0.05).

In Table 5, univariate analysis and subsequent multivariate analysis were conducted to identify the laboratory and clinical parameters independently associated with resistance to DAA therapy in subjects diagnosed...
with hepatitis C infection (non-responder and responder groups). The CXCR6 SNP dominant model (GG + GT) was significantly associated with DAA resistance (OR = 3.09, 95% CI: 1.33-7.16, p < 0.001).

**Discussion**

Oral DAAs are highly effective and safe, altering the disease burden in hepatitis C patients and its prognosis [14]. Many genome wide association studies have been performed to screen for host genetic variants associated with spontaneous clearance of HCV [12, 15], but in the DAA era there is a real need to conduct similar studies to determine host genetic polymorphisms in non-responders to DAA HCV treatment.

Considering CXCR6 (rs2234358) polymorphism, our study showed a statistically significant difference in distribution of the genotype and alleles frequencies between the three studied groups.

The findings suggest that the variant T allele is the major allele in the Egyptian population. In agreement with our findings, Picton et al. in 2017, after studying 232 South African healthy adult volunteers, reported that the T allele is the predominant allele in the South African population (G 40% and T 60%) [16], which is in contrast to the higher frequency of the G allele in the Chinese populations as reported by Chen et al. in 2017 [12].

The results suggested an association between the CXCR6 (rs2234358) alleles and the clearance of HCV infection after receiving DAAs. The allelic frequencies were 20% having the wild allele G and the remaining 80% having the variant allele T in the responder group, while in the non-responder group there were 39% with G and 61% with T alleles. This is consistent with what we mentioned before about the higher frequency of the T allele in our population and could explain to some extent the higher success rate of DAAs in the treatment of Egyptian HCV patients.

The comparison of genotype distribution and allelic frequencies of the CXCR6 SNP (rs2234358) between responder and non-responder groups revealed a significant risk of not responding to DAAs by 4.25 times for patients with the GG genotype versus TT genotype, while GT genotype was not significantly associated with the risk of not responding to DAAs as compared with TT genotype. Patients with the wild G allele showed a highly significant relation with 2.56 times risk of not responding to DAAs as compared with the variant allele T. Furthermore, the G allele showed a significant risk of not responding to DAAs in the dominant model (GG + GT vs. TT), but in the recessive model (GG vs. GT + TT) the risk narrowly missed statistical significance. Chen et al. reported that the T allele (minor in the Chinese population) was associated with clearance of HCV [12]. Our results revealed a protective effect of the T allele (major in the Egyptian population) that may explain the high prevalence of responding cases in HCV patients receiving DAAs in Egypt.

What could confirm the significant association between the T allele and a good virological response to DAA treatment when we compared the non-responder group with the control group. We found that GG genotype was significantly associated with the non-responding group by 5.5 times compared to the TT genotype and subjects with the wild G allele had a highly significant association with the non-responding group compared with the variant allele T by 2.56 times. The G allele also showed a highly significant association in the dominant model (GG + GT vs. TT) and similar results were obtained in the recessive model (GG vs. GT + TT). In contrast, the analysis of genotypes, alleles, and dominant and recessive models revealed no significant association between the responder and control groups.

We believe that our work is the first study to test the CXCR6 SNP (rs2234358) polymorphism in the Egyptian population. We explored its significant association with the clearance of HCV infection after receiving DAAs, revealed a significant risk of not responding to DAAs for patients with GG genotype versus TT genotype and also found that patients having the wild G allele showed a significant risk of not responding to DAAs as compared with the variant allele T.

Moreover, none of our patients had co-infection with HBV or HIV, were suffering liver cirrhosis or had other liver disease, so our results purely reflect the influence of the CXCR6 SNP (rs2234358) polymorphism on HCV response to treatment with DAAs.

However, we may need to conduct a similar study with a larger sample size to clearly validate our results and to explore other important SNP gene polymorphisms.

**Conclusions**

Direct acting antiviral agents are a breakthrough in the treatment of HCV due to the fewer side effects and shorter treatment duration with the higher response rates. The CXCR6 gene SNP (rs2234358) could have a potential role in virological response to DAA treatment with a protective effect of the T allele. There is a higher frequency of the T allele in our population, which could explain the higher observed success rate of DAAs in the treatment of Egyptian HCV patients.
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Disclosure

The authors declare no conflict of interest.

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