Al-Azhar International Medical Journal

Volume 4 | Issue 9 Article 1

2023

# Evaluation of Resistant Associated Variants / Substitutions (RAVs/RASs) Role in Management of Compensated Naive And Experienced Chronic Hepatitis C Virus Infection

Gamal Mohammad Mohammad Soliman
*Department of Tropical Medicine, Faculty of Medicine for Boys, Al-Azhar University, Cairo, Egypt*

Ashraf Taha Abd Elmouttaleb
*Medical Biochemistry and Molecular Biology, International Islamic Center - Al Azhar University, Cairo, Egypt.*

Mostafa Abd Elaziz Ahmed Abd Elrahman
*Department of Tropical Medicine, Faculty of Medicine for Boys, Al-Azhar University, Cairo, Egypt*

Mohamed Ghareb Mohamed Shikhroho
*Department of Tropical Medicine, Faculty of Medicine for Boys, Al-Azhar University, Cairo, Egypt*

Amr Mohammed Mohsen Aly Badary
*Department of Tropical Medicine,Police Authority Hospital, Cairo, Egypt*,amrbadary2001@yahoo.com



## How to Cite This Article

Soliman, Gamal Mohammad Mohammad; Elmouttaleb, Ashraf Taha Abd; Elrahman, Mostafa Abd Elaziz Ahmed Abd; Shikhroho, Mohamed Ghareb Mohamed; and Badary, Amr Mohammed Mohsen Aly (2023) "Evaluation of Resistant Associated Variants / Substitutions (RAVs/RASs) Role in Management of Compensated Naive And Experienced Chronic Hepatitis C Virus Infection," *Al-Azhar International Medical Journal*: Vol. 4: Iss. 9, Article 1.
DOI: https://doi.org/10.58675/2682-339X.2016

ORIGINAL ARTICLE

# Evaluation of Resistant Associated Variants/ Substitutions (RAVs/RASs) Role in Management of Compensated Naive and Experienced Chronic Hepatitis C Virus Infection

Gamal Mohammad Soliman [a], Ashraf Taha Abd Elmouttaleb [b], Mostafa Abd Abd Elrahman [a], Mohamed Ghareb Shikhroho [a], Amr Mohammed Badary [a,*]

[a] Department of Tropical Medicine, Faculty of Medicine for Boys, Al-Azhar University, Cairo, Egypt
[b] Medical Biochemistry and Molecular Biology, International Islamic Center, Al-Azhar University, Cairo, Egypt

**Abstract**

*Background*: **The success of DAA-based combination therapy for the treatment of HCV NS3 resistance-associated substitutions (RAS) is influenced by their occurrence.**

*Objective*: **The main aims of this study were (1) identifying the association of HCV relapse (posttreatment with ani-NS5A DAAs Simeprevir or Daclatasvir) with the occurrence of RAVs/RASs (resistance-associated substitutions) of nucleotide and specific amino acid replacement among Egyptians.**

*Methods*: **This cross-sectional, case−control study was conducted. Patients were enlisted by the Tropical Medicine. Department outpatient clinics at El-Hussein University (Al-Azhar University Faculty of Medicine) and EL-Agouza Police Hospital. In all, 150 HCV patients were involved in the study. These three groupings were created from them: 50 naive patients with chronic HCV infection comprise Group I and have compensated liver. (2) Group II: Fifty experienced patients (posttreatment with Sofosbuvir + Daclatasvir relapsers) who tested positive by serum HCV PCR after completing a full course of treatment and have compensated liver. (3) Group III: Fifty experienced patients (posttreatment with Sofosbuvir + Ladipasvir relapsers) who tested positive by serum HCV PCR after completing a full course of treatment and have compensated liver.**

*Results*: **There was no significant correlation found between HCV RNA PCR and HBA1C, FBS, INR, creatinine, bilirubin, albumin, AST, ALT, platelet, and hemoglobin in naïve patients ($P > 0.05$).**

*Conclusion*: **From the findings of the study, we can conclude that the frequency of L30L amino acid sequences was significantly higher in relapsers compared with responders and naïve patients. Responders showed significantly higher levels of L30L when compared with naïve patients.**

*Keywords:* Chronic hepatitis C virus, Infection, Management, Naïve, Resistance-associated variants, Substitutions (RAVs/RASs)

## 1. Introduction

Hepatitis C virus (HCV) infection is one of the world's most common causes of chronic liver damage. Three percent of the world's population, or roughly 130−210 million people, have HCV infection that is chronic.[1] According to another study presented at EASL 2014, there are 160 million chronic HCV-infected people globally.

There are considerable geographical variations. There are some nations like Egypt where the incidence is as high as 22 %.[2] According to more recent figures, 350,000 people die from HCV each year and more than 185 million people worldwide are

Accepted 23 February 2023.
Available online 7 November 2023

* Corresponding author at: Consultant of Gastroenterology and Hepatology, at Police Hospital Al-Agouza, Cairo, Egypt.
E-mail address: amrbadary2001@yahoo.com (A.M. Badary).

https://doi.org/10.58675/2682-339X.2016

affected. The majority of those infected with the virus are unaware of their infection, and treatment is still unavailable for many of those who have been diagnosed.[3] The majority of those treated find relief from their symptoms, and the success rates of those treated in low- and middle-income countries are comparable to those in high-income countries.[4] Cirrhosis of the liver or hepatocellular cancer is projected to occur in one-third of people who get a persistent infection.[5]

Egypt has the greatest prevalence in the world, with 9 % nationwide and up to 50 % in some rural regions as a result of particular mechanisms of infection. More than 150,000 new instances of infection are reported annually in Egypt, where 7 out of every 1000 people have HCV. Up to 91 % of Egyptians with HCV infection had genotype 4 as their main genotype, according to isolated samples.[6]

Patients with HCV genotype 4 infection have access to both IFN-containing and IFN-free treatment options. The IFN-free fixed-dose combination of Sofosbuvir (400 mg) and Ledipasvir (90 mg) in a single tablet taken once daily can be used to treat patients with HCV genotype 4 infection; 95 % of them had an SVR after 12 weeks of therapy.[7]

Low barriers to resistance exist for the HCV NS5A inhibitors Daclatasvir, Ledipasvir (LDV), and Ombitasvir. The results of two recent trials showed that failure to achieve SVR with DAA medication was usually due to relapse and that RAVs were almost invariably present in patients who relapsed after being subjected to NS5A inhibitors. In a study that looked at NS5A RAVs in patients who experienced virologic failure while taking Ledipasvir,[8] RAVs were almost always found. The single alterations at Q30E and Y93 N render NS5A inhibitors remarkably robust. It was unable to ascertain whether RAVs could be fought off with longer treatment intervals using the same regimen. Ledipasvir/Sofosbuvir-based regimens were supposed to be enrolled in the research and retreated for 24 weeks, yielding a 71 % SVR12, if they failed after 8 or 12 weeks. Sadly, the presence of certain NS5A RAVs, such as at the 93 locus, or the presence of several variations led to SVR rates of less than 50 %.[9]

The main aims of this study were (1) identifying the association of HCV relapse (posttreatment with ani-NS5A DAAs Simeprevir or Daclatasvir) with the occurrence of RAVs/RASs (resistance-associated substitutions) of nucleotide and specific amino acid replacement among Egyptians. (2) Recognizing the prevalence of the above-mentioned RAVs/RASs, resulting from nucleotide replacement and amino acid substitution in the NS5A region, at baseline pretreated naïve HCV infection. (3) Addressing the possible application of pretreatment screening for HCV RAVs/RASs that would help in considering the appropriate treatment regimens, duration of treatment, and avoidance of relapses.

## 2. Patients and methods

Patients were recruited from the Tropical Medicine Department outpatient clinics at El-Hussein University (Al-Azhar University Faculty of Medicine) and EL-Agouza Police Hospital.

**Grouping of Study Population:** The current study included a total of 150 HCV patients. The three groups they are split into are as follows: 50 patients in Group I who have chronic HCV infection but are unaware of it and have compensated liver. **Group II:** Fifty experienced patients (posttreatment with Sofosbuvir + Daclatasvir relapsers) who have tested positive by serum HCV PCR after completing a full course of treatment and have compensated liver. **Group III:** Fifty experienced patients (posttreatment with Sofosbuvir + Ladipasvir relapsers) who tested positive by serum HCV PCR after completing a full course of treatment and have compensated liver.

**Inclusion Criteria:** The following standard requirements will be met by all chosen patients: Age range of 18–70 years, third-generation enzyme-linked immunosorbent tests (ELISA), and polymerase chain reactions were utilized to identify positive anti-HCV IgG antibodies and serum HCV RNA, respectively. All research groups had a uniform Sex distribution (PCR). For the informed patients in groups II and III, a minimum 3-month course of sofosbuvir plus daclatasvir or sofosbuvir plus ledipasvir with or without ribavirin is necessary.

**Exclusion Criteria:** Exclusion criteria were applied while taking into account the Directives of the Egyptian National HCV Control Program. Patients with coinfection with HIV or HBV, those between the ages of 18 and 75 years, women who are pregnant, those with hepatocellular carcinoma or other extrahepatic malignancies, those with total serum bilirubin levels higher than 3 mg/dl, those with serum albumin levels lower than 2.8 g/dl, those with INR values higher than 1.7, those with platelet counts lower than 50,000/mm, those with renal impairment with GFR lower than 30 m, patients who refuse to be entitled in the study, other causes of chronic liver diseases (e.g. autoimmune hepatitis, biliary cirrhosis, cardiac cirrhosis, etc.), patients suffering from chronic uncontrolled debilitating disease, e.g. uncontrolled diabetes Mellitus, sarcoidosis, SLE, etc., posttreatment patients who had one of the major risk factors of HCV reinfection within 3 months after completing treatment course,

e.g. dental work up, minor surgery, anorectal surgery, blood transfusion, i.v. drug abuse etc, patients receiving immunosuppressive or cytotoxic medicine, and patients present with decompensated hepatic disorders.

### 2.1. Methods

All patients were subjected to the following:

**Full history taking** including risk factors of occurrence of HCV (newly discovered right hypochondrial pain, parentral antibilharzial therapy, blood transfusion, and previous operation).

**Thorough clinical examination** with special emphasis on signs of liver cell failure; hepatomegaly, splenomegaly, and/or ascites.

**Laboratory investigations:** Patients' blood was drawn, and samples were sent to the following laboratories: The hemoglobin concentration (Hb%), red blood cells (RBCs), white blood cells (WBCs), and platelet counts are among the measurements included in the complete blood count (CBC). In addition, the liver profile (ALT, AST, albumin, total bilirubin, and direct bilirubin), prothrombin time, the international normalized ratio (INR), renal function tests (serum creatinine), pregnancy tests (for females), and fasting blood glucose level.

**Imaging:** Study patients were submitted to screening with the following procedures: Abdominal ultrasonography which includes liver size (diameter), liver echogenicity, portal and splenic vein diameters, splenic size, and amount of ascites if present. Pretreatment fibrous assessment of the liver by transient elastography (FibroScan).

**Nucleotide Sequences:** The isolated RNA from patients' sera will be submitted to nucleotide sequencing at NS5A areas to address the base pair (bp) differences among HCV isolates from different study groups in comparison to gold standard HCV GT4.

### 2.2. The expected study outcomes

Treatments of chronic HCV infections with anti-HCV regimens that contain DAAs alone or with regimens that add Ribavirin to DAAs have shown high cure rates that exceeded 80 % in some series. It is expected that data from current study will reveal the following:- The expected occurrence of RAVs/RASs in association with anti-NS5A agents in GT4 would be close to their prevalence in GT1. The degree of hepatic fibrosis would play a negative role in outcomes of the relationship between RAVs/RASs and the studied therapeutic regimens. A higher degree of hepatic fibrosis would be associated with a higher prevalence of RAVs/RASs in posttreatment relapsers. RAVs/RASs would have the same occurrence rates in Daclatasvir compared with Ledipasvir-containing regimens. A lower prevalence of RAVs/RASs would be expected on adding Ribavirin to Sofosbuvir plus Daclatasvir or Sofosbuvir plus Ledipasvir compared with the use of only DAAs containing regimens without Ribavirin.

**Ethical Aspects: Approving protocol:** The current protocol will be approved by the Committee of Tropical Medicine Department and Committee of Faculty of Medicine at Al-Azhar University, and then by the ethics committee at Al-Azhar University. **Permission from El Agouza Police Hospital: -** Permission will be obtained from El-Agouza Police Hospital to recruit study subjects from the outpatient HCV management clinics and to prospectively follow them up. **Patient Consent:** All patients that were included in the current study signed an approved consent from the Al-Azhar University Ethics Committee.

### 2.3. Statistical analysis

The gathered information will be identified, collated, and examined using proper tests. Mean and standard deviation are used to represent continuous variables, whereas numbers and percentages are used to describe ordinal and nominal categorical data.

## 3. Results

The present study included 150 HCV patients recruited from the Tropical Medicine Department outpatient clinics at El-Hussein University (Al-Azhar University Faculty of Medicine) and EL-Agouza Police Hospital Table 1.

This table shows the demographic of the included cases. The mean age was 44.97 ± 11.88 years with a range from 25 years to 64 years. More than half (50.5 %) of cases were females and 49.5 % of cases were males Table 2.

There were no high significant differences found between the studied groups concerning HCV RNA PCR level at baseline ($P = 0.001$) (see Tables 3–7).

*Table 1. Distribution of study population according to baseline characteristics.*

| | (Min–max) | Mean ± SD |
|---|---|---|
| Age | (25–64) | 44.97 ± 11.88 |
| Sex* | **Male** 49 | (49.5) |
| | **Female** 50 | (50.5) |

*Table 2. Relationship between different study groups and HCV RNA PCR at baseline.*

| Groups | N | (min–max) | mean ± SD | 95 % CI | *P* value |
|---|---|---|---|---|---|
| Naïve | 33 | (203–1480000) | 286613.2 ± 384696.8 | (150205.7–423020.8) | |
| Responder | 52 | (0–15) | 1.15 ± 3.75 | (0.11–2.2) | 0.001[a] |
| Relapser | 14 | (1000–600000) | 100357.1 ± 172403.2 | (814.5–199899.8) | |
| Total | 99 | (0–1480000) | 109730.3 ± 263065.8 | (57262.7–162197.8) | |

[a] Statistically significant.

*Table 3. Post hoc test.*

| | Mean difference | *P* value |
|---|---|---|
| Naïve | | |
| Responder | 286612.06 | 0.001* |
| Relapser | 186256.07 | 0.013 * |

There was a highly statistically significant difference **(*P* = 0.001)** between the naïve group and the responder group. A statistically significant difference **(*P* = 0.013)** was seen between the naïve group and the relapser group.

*Table 4. Relationship between different study groups and FibroScan score at baseline.*

| | Naïve *N* (%) | Responder *N* (%) | Relapser *N* (%) | *P* value |
|---|---|---|---|---|
| 0 | 14 (42.4) | 11 (21.2) | 7 (50.0) | |
| 1 | 6 (18.2) | 10 (19.2) | 4 (28.6) | |
| 2 | 7 (21.2) | 15 (28.8) | 1 (7.1) | 0.17 |
| 3 | 6 (18.2) | 16 (30.8) | 2 (14.3) | |
| Total | 33 (100) | 52 (100) | 14 (100) | |

There was no significant differences found between the studied groups concerning the FibroScan score at baseline ($P > 0.05$).

This table shows:

There was no significant correlation found between HCV RNA PCR and HBA1C, FBS, INR, creatinine, bilirubin, albumin, AST, ALT, platelet, and hemoglobin in naïve patients ($P > 0.05$).

There was a significant negative correlation between HCV RNA PCR and albumin ($r = -0.29$, $P = 0.04$), while there was a significant positive correlation between HCV RNA PCR and hemoglobin ($r = 0.32$, $P = 0.02$) in patients with a sustained virological response. No significant correlation was found between HCV RNA PCR and HBA1C, FBS, INR, creatinine, bilirubin, AST, ALT, and platelet in patients with sustained virological response ($P > 0.05$).

*Table 5. Correlation between HCV RNA PCR and different laboratory findings at baseline regarding naïve patients.*

| HCV RNA PCR (mean ± SD) | Laboratory test | mean ± SD | R | *P* value |
|---|---|---|---|---|
| (286613.21 ± 384696.75) | HBA1C | 5.19 ± 0.24 | −0.05 | 0.79 |
| | FBS | 103.9 ± 15.02 | −0.2 | 0.26 |
| | INR | 1.35 ± 0.09 | 0.06 | 0.73 |
| | Creatinine | 0.98 ± 0.12 | 0.15 | 0.4 |
| | Bilirubin | 1.72 ± 0.44 | 0.04 | 0.84 |
| | Albumin | 4.21 ± 0.45 | 0.05 | 0.78 |
| | AST | 70.72 ± 22.01 | −0.13 | 0.48 |
| | ALT | 77.24 ± 19.59 | 0.05 | 0.79 |
| | Platelet | 177.98 ± 37.23 | 0.17 | 0.33 |
| | Hemoglobin | 12.14 ± 0.6 | 0.01 | 0.94 |

*Table 6. Correlation between HCV RNA PCR and different laboratory findings at baseline regarding patients with sustained virological response.*

| HCV RNA PCR (mean ± SD) | Laboratory test | mean ± SD | R | *P* value |
|---|---|---|---|---|
| (1.15 ± 3.75) | HBA1C | 1.15 ± 3.75 | −0.15 | 0.28 |
| | FBS | 5.21 ± 0.22 | −0.15 | 0.28 |
| | INR | 105.16 ± 14.52 | 0.11 | 0.43 |
| | Creatinine | 1.35 ± 0.08 | −0.03 | 0.84 |
| | Bilirubin | 1 ± 0.12 | 0 | 0.98 |
| | Albumin | 1.67 ± 0.42 | −0.29 | 0.04[a] |
| | AST | 4.21 ± 0.43 | −0.02 | 0.88 |
| | ALT | 71.78 ± 22.06 | 0.06 | 0.67 |
| | Platelet | 71.26 ± 17.63 | −0.08 | 0.58 |
| | Hemoglobin | 170.52 ± 47.45 | 0.32 | 0.02[a] |

[a] Statistically significant.

## 4. Discussion

Their existence affects the efficacy of DAA-based combination therapy for the treatment of HCV NS3 resistance-associated substitutions (RAS). Individuals with mutations at NS3 sites 80, 122, 155,

*Table 7. Correlation between HCV RNA PCR and different laboratory findings at baseline regarding patients with breakthrough after treatment.*

| HCV RNA PCR (mean ± SD) | Laboratory test | mean ± SD | R | *P* value |
|---|---|---|---|---|
| (100357.14 ± 172403.2) | HBA1C | 5.15 ± 0.19 | 0.32 | 0.27 |
| | FBS | 108.51 ± 15.46 | 0.18 | 0.53 |
| | INR | 1.35 ± 0.09 | 0.35 | 0.22 |
| | Creatinine | 0.98 ± 0.1 | −0.27 | 0.36 |
| | Bilirubin | 1.71 ± 0.43 | −0.49 | 0.07 |
| | Albumin | 4.37 ± 0.33 | −0.42 | 0.14 |
| | AST | 74.57 ± 18.14 | 0.11 | 0.72 |
| | ALT | 70 ± 18.52 | 0.09 | 0.76 |
| | Platelet | 193.64 ± 41.7 | −0.19 | 0.5 |
| | Hemoglobin | 12.02 ± 0.6 | 0.19 | 0.51 |

There was no significant correlation between HCV RNA PCR and HBA1C, FBS, INR, creatinine, bilirubin, albumin, AST, ALT, platelet, and hemoglobin in patients with breakthrough after treatment ($P > 0.05$).

and/or 168 did not respond to Dackltasvir/Simeprevir therapy.

The frequency of spontaneously occurring single RASs in HCV genotype 1-infected patients ranges from 0.1 % to 3.1 % for the majority of NS3 protease inhibitors and 4.1 %–18.9 % of HCV-infected patients with baseline NS3 mutations exhibit naturally occurring resistance.[10]

This a cross-sectional, case–control study. Patients were gathered from El-Hussein University's (Al-Azhar University Faculty of Medicine) outpatient clinics for tropical medicine and the EL-Agouza Police Hospital. In all, 150 HCV patients were involved in the study. These three groupings were created from them: 50 naive patients with chronic HCV infection comprise Group I and have compensated liver. 2) Group II: Fifty experienced patients (post-treatment with Sofosbuvir + Daclatasvir relapsers) who tested positive by serum HCV PCR after completing a full course of treatment and have compensated liver. 3) Group III: Fifty experienced patients (posttreatment with Sofosbuvir + Ledipasvir relapsers) who tested positive by serum HCV PCR after completing a full course of treatment and have compensated liver.

As regards the demographic of the included cases, the mean age was 44.97 ± 11.88 years with a range from 25 years to 64 years. More than half (50.5 %) cases were females, and 49.5 % cases were males.

Our results were supported by a study of **El Raziky *et al.***[11] According to their research, the patients' ages ranged from 36 to 70 years with a mean age of 55.83 years plus 7.9. Sixty-eight percent of the study's patients were female. Furthermore, Kanda et al.[12] showed that 145 (60.4 %) people were 65 years of age or older and that the mean age was 65.8 years. They were mainly composed of females. The baseline HCV RNA PCR levels between the study groups showed highly significant variations ($P$ 0.001). The post hoc analysis revealed a very statistically significant difference between the naive group and the responder group ($P = 0.001$). A statistically significant difference exists between the naive group and the relapser group ($P = 0.013$). HCV RNA PCR results in naive patients did not significantly correlate with HBA1C, FBS, INR, creatinine, bilirubin, albumin, AST, ALT, platelet, or hemoglobin ($P > 0.05$). In patients with persistent virological response, there was a substantial negative connection between HCV RNA PCR and albumin ($r = -0.29$, $P = 0.04$) and a significant positive association between HCV RNA PCR and hemoglobin ($r = 0.32$, $P = 0.02$). In patients with persistent virological response, there was no association between HCV RNA PCR and HBA1C, FBS, INR, creatinine, bilirubin, AST, ALT, or platelet counts ($P > 0.05$). In patients with treatment-related breakthrough, there was no association between HCV RNA PCR and HBA1C, FBS, INR, creatinine, bilirubin, albumin, AST, ALT, platelet, or hemoglobin ($P > 0.05$).

Our results were supported by a study by **NASSAR *et al.***,[13] which reported that regarding efficacy assessment, the SVR rates for the three groups (SOF/DCVRBV, SOF/LDVRBV, and SOF/SIMRBV) were 98 %, 100 %, and 100 %, respectively.

Only one patient—a member of the SOF/DCV group—experienced virological failure. Statistics indicated that these findings were not significant ($P > 0.05$. The baseline variables, such as regimen type, patient sex, treatment status, baseline viral load, platelet count, and cirrhosis status, did not statistically significantly predict SVR. In the study of **Abd Alla *et al.***,[14] liver fibrosis was associated with a significant decrease in serum albumin, hemoglobin, platelet count, and white blood cells and a significant increase in INR and serum bilirubin compared with hepatic fibrosis-free patients. Furthermore, **Abdel-Aziz *et al.***[15] examined the effectiveness and safety of treating HCV genotype 4 patients with sofosbuvir plus daclatasvir with or without ribavirin. They discovered that group 1 had a sustained virological response (SVR1) of 93.3 % and group 2 had an SVR12 of 87.5 % (total = 91 %; undetectable viremia 12 weeks posttreatment). With such great efficiency came moderate side effects as well as a considerable reduction in liver fibrosis. The accomplishment of SVR12 in HCV patients following therapy was not significantly correlated with the IL18 polymorphism (rs1946518) at position 607 in these individuals. Furthermore, 249 (12.2 %) of 2047 people who underwent hepatitis C virus screening received positive results, according to Ahmed et al. 88.7 % of the 249 people, or 221 of them, had RNA that could be found by PCR. At week 12, 183 eligible patients receiving Sofosbuvir and Daclatasvir for 12 weeks showed a sustained virologic response, accounting for 96 % of the total. Moreover, Abd Alla et al.[14] discovered that SOF plus DCV regimens with or without ribavirin led to a 12-week SVR in 98 % of the patients with chronic HCV infection. Diabetes mellitus and hepatic fibrosis had detrimental consequences on 12-week SVR. Despite baseline viral loads not having an impact on the effectiveness of anti-HCV oral medication, F4 hepatic fibrosis is linked to the highest pretreatment HCV-SRT-PCR levels. The present study showed the relationship between the administration of SOF + LAD regimen for 12 weeks and development of aa sequences in relapsers and responders who

achieved SVR. None of the studied aa substitutions (M31 M, A92A, M31C, A92T, P58L) was detected in naïve infection. Both M31 M and A92A aa sequences were significantly recognized in relapsers and responders compared with naïve cases. However, frequencies of the same aa substitutions (M31 M and A92A) had insignificant differences in relapsers compared with responders.

In the study of **Sarrazin *et al.*,**[16] in 11 phase 3 clinical trials using ledipasvir-sofosbuvir (SOF) and SOF, 12 weeks following the completion of treatment, just 12 of 3004 patients still had detectable HCV RNA. The HCV genotype/subtype was the same at baseline and at the time of recurrence in 11 of the 12 patients with late recurrent viremia. According to phylogenetic research, the SOF-based regimen successfully eliminated HCV in 58 % (7 of 12) of these patients; however, after therapy, they returned with a new HCV strain. The HCV that was identified at baseline lingered in the liver or another compartment and reappeared in the blood 24 weeks after treatment in the remaining five patients with late recurrent viremia. Furthermore, Osinusi et al.,[17] in previously untreated patients with HCV genotype 1 and HIV coinfection, assess the rates of sustained virologic response (SVR) and side effects after a 12-week course of the fixed-dose combination of ledipasvir and sofosbuvir. It was claimed that 48 out of 50 patients (98 %, 95 % CI, 89%−100 %) had achieved SVR 12 weeks following the completion of treatment, but one patient experienced a recurrence at week 4 after concluding the course of treatment. Deep sequencing discovered a mutation that conferred resistance to NS5A inhibitors like ledipasvir in the NS5A region of the patient who had relapsed. Myalgia (14 % of patients) and nasal congestion (16 % of patients) were the most typical side effects. There were no major side effects or discontinuations linked to the trial medication. In patients who are carefully chosen, Kowdley et al.[18] showed that an 8-week LDV/SOF therapy course is highly beneficial; more frequent use of this regimen is advised. Afdhal et al. found that 79 % of the 440 patients who got therapy and 20 % of the 440 randomly assigned patients had HCV genotype 1a infection and 72 % had cirrhosis. High rates of long-lasting virologic response were seen in all therapy groups, including 99 % (95 % CI, 95−100) in the group receiving 24 weeks of ledipasvir-sofosbuvir, 94 % (95 % confidence interval [CI], 87−97) in the group receiving 12 weeks of ledipasvir-sofosbuvir, and 96 % (95 % CI, 91 to 99) in the group receiving 12 weeks of ledipasvir-sofosbuvir; 41 of 50 HCV genotype 3 patients who had undergone therapy had an SVR12 (82 %) rate. SVR12 rates were 96 % for patients with HCV genotype 6 out of 25 patients. Furthermore, in the trial by Mizokami et al.,[19] all 171 (100 %) of the patients getting ledipasvir-sofosbuvir and 167 (98 %) of 170 patients receiving ledipasvir-sofosbuvir with ribavirin achieved the SVR12 (95 % CI 95−100) (80 of 83 treatment-naive and 87 of 87 treatment-experienced). SVR12 was present in 75 (99 %) of the 76 people who had NS5A-resistant mutations at baseline.

### *4.1. Conclusion*

From the findings of the study, we can conclude that the frequency of L30L amino acid sequences was significantly higher in relapsers compared with responders and naïve patients. Responders showed significantly higher levels of L30L when compared with naïve patients. The study showed the relationship between the administration of SOF + LAD regimen for 12 weeks and development of aa sequences in relapsers and responders who achieved SVR. None of the studied aa substitutions (M31 M, A92A, M31C, A92T, P58L) was detected in naïve infection. Both M31 M and A92A aa sequences were significantly recognized in relapsers and responders compared with naïve cases. However, frequencies of the same aa substitutions (M31 M and A92A) had insignificant differences in relapsers compared with responders.

## Disclosure

The authors have no financial interest to declare in relation to the content of this article.

## Authorship

All authors have a substantial contribution to the article.

## Sources of funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

## Conflicts of interest

The authors declared that there were no conflicts of interest.

## References

1. Lavanchy D. The global burden of hepatitis C. *Liver Int*. 2009; 29:74−81.
2. World Health Organization. *Factsheet No 164*. June 2011.

3. Lemoine M, Nayagam S, Thursz M. Viral hepatitis in resource-limited countries and access to antiviral therapies: current and future challenges. *Future Virol*. 2013;8:371–380.
4. Ford N, Kirby C, Singh K. Chronic hepatitis C treatment outcomes in low- and middle-income countries: a systematic review and meta-analysis. *Bull World Health Organ*. 2012;90: 540–550.
5. Ly KN, Xing J, Klevens RM. The increasing burden of mortality from viral hepatitis in the United States between 1999 and 2007. *Ann Intern Med*. 2012;156:271–278.
6. Miller D, Abu-Raddad L. Evidence of intense ongoing endemic transmission of hepatitis C virus in Egypt. *Proc Natl Acad Sci*. 2010;107:14757–14762.
7. Kapoor R, Kohli A, Sidharthan S. All-oral treatment for genotype 4 chronic hepatitis C infection with sofosbuvir and ledipasvir: interim results from the NIAID SYNERGY trial. *Hepatology*. 2014;60:321A.
8. El-Shamy A, Shoji I, Kim SR, et al. Sequence heterogeneity in NS5A of hepatitis C virus genotypes 2a and 2b and clinical outcome of pegylated-interferon/ribavirin therapy. *PLoS One*. 2012;7:e30513.
9. Lawitz E, Flamm S, Yang JC, et al. Retreatment of patients who failed 8 or 12 weeks of ledipasvir/sofosbuvir-based regimens with ledipasvir/sofosbuvir for 24 weeks. In: *Presented at the European Association for the Study of the Liver 50th International Liver Congress. Vienna, Austria*. 2015;62:22–26.
10. Lenz O, Verbinnen T, Fevery B, et al. Virology analyses of HCV isolates from genotype 1-infected patients treated with simeprevir plus peginterferon/ribavirin in Phase IIb/III studies. *J Hepatol*. 2015;62:1008–1014.
11. El Raziky M, Hamdy S, Hamada Y, et al. Efficacy and safety of sofosbuvir and daclatasvir in patients with chronic hepatitis C virus induced cirrhosis with Child-Pugh class B. *Egypt Liver J*. 2022;12:1–9.
12. Kanda T, Yasui S, Nakamura M, et al. Real-world experiences with the combination treatment of ledipasvir plus sofosbuvir for 12 weeks in HCV genotype 1-infected Japanese patients: achievement of a sustained virological response in previous users of peginterferon plus ribavirin with HCV NS3/4A inhibitors. *Int J Mol Sci*. 2017;18:906.
13. Nassar AK, Al-Sayed A, Abd El-Raouf HS, Eid BM. Efficacy, safety and relapse of sofosbuvir in combination with daclatasvir, ledipasvir and simeprevir for treatment of hepatitis C virus in Egypt. *Med J Cairo Univ*. 2018;86:2223–2230. l.
14. Abd Alla MD, Gomaa AA, Abou Farrag GA, et al. Retrospective study of hepatitis c virus relapse after treatment with sofosbuvir and daclatasvir with or without ribavirin. *Al-Azhar Assiut Med J*. 2018;16:197.
15. Abdel-Aziz AM, Ibrahim MA, El-Sheikh AA, et al. Effect of sofosbuvir plus daclatasvir in hepatitis C virus genotype-4 patients: promising effect on liver fibrosis. *J Clin Exp Hepatol*. 2018;8:15–22.
16. Sarrazin C, Isakov V, Svarovskaia E, et al. Late relapse versus HCV reinfection in patients with sustained virologic response after sofosbuvir-based therapies. *Clin Infect Dis*. 2016:ciw676.
17. Osinusi A, Townsend K, Kohli A, et al. Virologic response following combined ledipasvir and sofosbuvir administration in patients with HCV genotype 1 and HIV co-infection. *JAMA*. 2015;313:1232–1239.
18. Kowdley KV, Sundaram V, Jeon CY, et al. Eight weeks of ledipasvir/sofosbuvir is effective for selected patients with genotype 1 hepatitis C virus infection. *Hepatology*. 2017;65: 1094–1103.
19. Mizokami M, Yokosuka O, Takehara T, et al. Ledipasvir and sofosbuvir fixed-dose combination with and without ribavirin for 12 weeks in treatment-naive and previously treated Japanese patients with genotype 1 hepatitis C: an open-label, randomised, phase 3 trial. *Lancet Infect Dis*. 2015;15:645–653.