Hepatitis C virus recurrence in two patients who achieved sustained viral response with interferon-free direct-acting antiviral therapy: reinfection or relapse?

Hepatitis C virus recurrence in two patients who achieved sustained viral response with interferon-free direct-acting antiviral therapy: reinfection or relapse?

Yoshimi Yukawa, Akihiro Tamori, Etsuko Iio, Shintaro Ogawa, Kanako Yoshida, Sawako Uchida-Kobayashi, Masaru Enomoto, Yasuhito Tanaka, Norifumi Kawada

Citation	Clinical Journal of Gastroenterology. 12(6); 598–602
Issue Date	2019-12
Published	2019-06-04
Type	Journal Article
Textversion	Author
Rights	This is a post-peer-review, pre-copyedit version of an article published in Clinical Journal of Gastroenterology. The final authenticated version is available online at: https://doi.org/10.1007/s12328-019-01001-w. See Springer Nature terms of use. https://www.springer.com/gp/open-access/publication-policies/aam-terms-of-use.
DOI	10.1007/s12328-019-01001-w

Yukawa, Y., Tamori, A., Iio, E. et al. Hepatitis C virus recurrence in two patients who achieved sustained viral response with interferon-free direct-acting antiviral therapy: reinfection or relapse? *Clinical Journal of Gastroenterology*. 12, 598–602 (2019). Doi: 10.1007/s12328-019-01001-w

Hepatitis C virus recurrence in two patients who achieved sustained viral response with interferon-free direct-acting antiviral therapy: reinfection or relapse?

Running title: HCV relapse or reinfection after DAA therapy

Yoshimi Yukawa, Akihiro Tamori, Etsuko Iio*, Shintaro Ogawa*, Kanako Yoshida, Sawako Uchida-Kobayashi, Masaru Enomoto, Yasuhito Tanaka*, Norifumi Kawada

Department of Hepatology, Graduate School of Medicine, Osaka City University, Osaka, Japan

*Department of Virology & Liver Unit, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan

Corresponding Author: Akihiro Tamori M.D., PhD.

Department of Hepatology, Blood Transfusion Unit, Osaka City University Graduate School of Medicine, 1-4-3, Asahimachi, Abeno-ku, Osaka, 5458585, JAPAN

Tel; +81-6-6645-2292

Fax; +81-6-6646-1433

e-mail: atamori@med.osaka-cu.ac.jp

ABSTRACT

We experienced two patients with chronic hepatitis C (HCV) in whom it was difficult to distinguish between relapse and reinfection after interferon-free direct-acting antiviral (DAA) therapy. Case 1 was a 55-year-old man infected with HCV genotype 1b, at 5.6 log IU/mL, with a history of injecting drug use. He was treated with ombitasvir (25 mg), paritaprevir (150 mg), and ritonavir (100 mg) for 12 weeks. After DAA therapy, recurrent HCV showed that genotype 2a differed from the baseline genotype. Close examination of the baseline sample showed that he was coinfected with HCV genotypes 1b and 2a. We confirmed that the recurrent 2a HCV had a resistant associated substitution to ombitasvir. Case 2 was a 60-year-old woman infected with HCV genotype 2b, at 5.7 log IU/mL. She was treated with sofosbuvir (400 mg) and ribavirin (600 mg) for 12 weeks and achieved a sustained virological response (SVR) at 24 weeks. Even after SVR24, her serum levels of alanine aminotransferase (ALT) fluctuated. HCV RNA was detected again 48 weeks after the end of treatment. She had a sexual partner who was also infected with HCV2b. The phylogenetic tree analysis revealed a high degree of homology among the three strains, pre- and post-HCV treatment, and her partner. After recurrence of HCV, the HCV RNA level decreased spontaneously to below the limit of detection and ALT levels normalized. Her genotype of *Interleukin 28B* was major. It is important to make a precise diagnosis regarding reemerged HCV after DAA therapy.

Key Words: direct-acting antiviral, hepatitis C virus, late relapse, reinfection, sustained virological response

Abstract: 247 words

Text: 1313 words

1 Table and 2 Figures

INTRODUCTION

The development of direct-acting antivirals (DAAs) has markedly improved the rate of viral eradication in patients infected with chronic hepatitis C virus (HCV). However, HCV can still recur and it is important to distinguish between reinfection with HCV after achieving a sustained virological response (SVR) and late viral relapse. In the era of interferon-based therapy, late relapse was reportedly rare,[1] and testing for an SVR 12 weeks after completion of therapy (SVR12) is as effective at predicting SVR as testing at 24 weeks (SVR24).[2] The European Association for the Study of the Liver (EASL) guidelines recommend that patients who achieve SVR should be retested for HCV RNA 48 weeks after completing treatment, and that the infection can be considered definitely cured when HCV RNA is undetectable up to that time. It has been recommended to monitor HCV reinfection with an annual HCV RNA assessment in high-risk patients; such patients include injecting drug users (IDUs), men who have sex with men, and those engaging in other high-risk behaviors.[3] The American Association for the Study of Liver Diseases (AASLD) guidelines recommend assessing HCV relapse or reinfection in patients at risk for HCV infection, or with unexplained hepatic dysfunction.[4] Higher rates of reinfection may be expected in the era of DAA treatment due to increasing treatment access for those with ongoing high-risk behavior and reduced concerns over the adverse effects of treatment.[5] Between September 2014 and November 2018, 689 patients (mean age, 67 ± 12 years; 309 males and 380 females)

treated with DAAs were followed up beyond 48 weeks after the end of treatment. We experienced two cases of suspected reinfection among 16 non-SVR patients.

CASE REPORT

CASE 1

A 55-year-old man was infected with HCV genotype 1b, at 5.6 log IU/mL. Laboratory data at baseline are shown in Table. He had been an IDU. He was treated with ombitasvir (25 mg), paritaprevir (150 mg), and ritonavir (100 mg) for 12 weeks. Although HCV RNA was undetectable 4 weeks after starting treatment and SVR4 was achieved, HCV RNA reappeared 12 weeks after the end of treatment. The HCV genotype at treatment failure was 2a which was different from the baseline genotype. Phylogenetic analysis of NS5B also revealed that the HCV genotype at treatment failure differed from that at baseline. These results strongly suggest that this patient had been reinfected with another HCV genotype after the initial DAA therapy. However, the existence of two HCV types at baseline was revealed after separating several clones amplifying them with the conventional method, and analyzing the sequences (Fig. 1A). We concluded that the ombitasvir/paritaprevir/ritonavir therapy eradicated genotype 1b HCV, but genotype 2a HCV remained. We confirmed the emergence of a resistance-associated substitution in NS5A of genotype 2a after the initial DAA therapy. Retreatment with glecaprevir (100 mg) and pibrentasvir (40 mg) for 12 weeks achieved SVR12 (Fig. 1B).

CASE 2

A 60-year-old woman was infected with HCV genotype 2b, at 5.7 log IU/mL (Table). She did not have a history of injecting drug use and a family history for HCV. The *IL28B* rs8099917 single nucleotide polymorphism (SNP) was the major type, TT.[6] She was treated with sofosbuvir (400 mg) and ribavirin (600 mg) for 12 weeks and achieved SVR24. Even after SVR24, her serum levels of alanine aminotransferase (ALT) remained elevated. HCV RNA was detected again at 48 weeks after the end of treatment (Fig. 2A). She had a sexual partner who was also infected with HCV. To discriminate between relapse and reinfection from the partner, we compared the HCV NS5B sequences of three samples: her HCV at baseline and at treatment failure, and her partner's HCV. Phylogenetic tree analysis based on HCV NS5B sequences spanning 339 nt (nucleotide position: 8278-8618) was performed. The analysis revealed a high degree of homology among the three strains; pre- and post- HCV treatment, and her partner (Fig. 2B). It was difficult to differentiate between reinfection and late relapse using HCV sequence analysis. After recurrence of HCV, the HCV RNA level decreased spontaneously to below the limit of detection at 24 weeks after the repeat detection and ALT levels normalized, similar to the typical clinical course of HCV infection-induced acute hepatitis. Her partner achieved SVR treated with a combination of sofosbuvir and ribavirin therapy after recurrence of HCV.

DISCUSSION

We presented two patients who were suspected HCV reinfection after interferon-free DAA therapy. In case 1, the HCV genotype at baseline differed from that at failure. In addition, the patient had a history of IDU. *Midgard* et al. reported that HCV reinfection is common, with an incidence of 1.7/100 person-years (95% CI 0.8–3.1) among individuals who returned to IDU after treatment.[5] Therefore, we strongly suspected HCV reinfection in this case. However, sub-cloning analysis revealed coinfection with HCV genotypes 1b and 2a at baseline. Combination therapy with ombitasvir/paritaprevir/ritonavir for DAA naïve patients with HCV genotype 1 achieves a high SVR rate.[7] However, a phase II trial that used these DAAs for 12 weeks reported a SVR 12 rate of 72% for HCV genotype 2-infected Japanese. It was speculated that this regimen was not effective for patients with genotype 2, although the base HCV viral load was low.[8] Now a combination with the pan-genotypic regimen glecaprevir/pibrentasvir is available as one of the first recommended options in Japan. No study has reported that one of the coinfected HCV emerged after pan-genotypic DAA therapy yet. *Okamoto et al.* reported that 4 (1.6%) of 256 patients were infected with two different HCV genotypes.[9] It is important to note that IDUs have the opportunity for infection with more than one HCV genotype. There are three patterns of HCV RNA recurrence in SVR patients: relapse, persistence/emergence of a pre-existing minority variant, and reinfection.[10] Case 1 presented herein corresponded to the emergence of a pre-existing minority variant: HCV genotype 2a.

In case 2, HCV RNA was detected again 48 weeks after the end of sofosbuvir/ribavirin treatment. The ALT levels fluctuated continuously during the SVR. Therefore, we tested for HCV RNA repeatedly. Serum levels of ALT increased abruptly and HCV RNA became detectable following SVR48. A late relapse was suspected. However, the patient had an HCV-infected sexual partner, whose HCV RNA sequence showed a high degree of homology to those of the patient at baseline and reemergence. The same pathogen can pass to and from patients in sexually transmitted infections. In two prospective chort studies of monogamous heterosexual couples, the incidence rate of HCV infection in seronegative partners with chronic HCV infection was 0-0.6%. [11,12] Although sexual transmission of HCV was reported to be extremely rare, we could not distinguish between late relapse and reinfection based on a comparison of HCV RNA sequences. The reemerged HCV disappeared spontaneously, similar to the clinical course of acute hepatitis C. HCV infection is often self-limited in patients with the *IL28B* major type.[13] Therefore, we strongly suspect that our patient had been reinfected with HCV by her partner.

No patient in the cohort seen at our university hospital had a definite HCV reinfection after DAA therapy. Across 11 phase III clinical trials of sofosbuvir-based regimens, only 12 of 3,004 patients (0.40%) had detectable HCV RNA following SVR12. Of these 12 patients, 7 were reinfected with a different HCV genotype after treatment.[14] Most studies have analyzed recurrence after treatment with interferon-based therapy, and there is no evidence to support the hypothesis that recurrence rates differ in DAA-based

treatment regimens.[1] In the era of DAA treatment, resistance-associated substitutions have been detected in relapsed HCV, apart from natural HCV. It is necessary to distinguish between relapse and reinfection to determine the DAA regimen for retreatment.

In conclusion, we encountered one case of coinfection with two HCV genotypes and another with a high possibility of reinfection following a DAA-based treatment. It is important to make a precise diagnosis regarding reemerged HCV after DAA therapy.

Funding

None declared.

Conflicts of Interest

The authors have no conflicts of interest.

REFERENCES

1. Simmons B, Saleem J, Hill A, Riley RD, Cooke GS. Risk of late relapse or reinfection with hepatitis C virus after achieving a sustained virological response: A systemic review and meta-analysis. *Clin Infect Dis* 2016; 62: 683–694.

2. Marinot-Peignoux M, Stern C, Maylin S, et al. Twelve weeks posttreatment follow-up is as relevant as 24 weeks to determine the sustained virologic response in patients with hepatitis C virus receiving pegylated interferon and ribavirin. *Hepatology* 2010; 51: 1122–1126.

3. European Association for the Study of the Liver. EASL recommendations on treatment of hepatitis C 2016. *J Hepatol* 2017; 66: 153–194.

4. AASLD/IDSA HCV Guidance Panel. Hepatitis C guidance: AASLD-IDSA recommendations for testing, managing, and treating adults infected with hepatitis C virus. *Hepatology* 2015; 62: 932–954.

5. Midgard H, Bjøro B, Mæland A, et al. Hepatitis C reinfection after sustained virological response. *J Hepatol* 2016; 64: 1020–1026.

6. Tanaka Y, Nishida N, Sugiyama M, et al. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. *Nat Genet* 2009; 41: 1105–1109.

7. Kumada H, Chayama K, Rodrigues L Jr, et al. Randomized phase 3 trial of ombitasvir/paritaprevir/ritonavir for hepatitis C virus genotype 1b-infected Japanese patients with or without cirrhosis. *Hepatology* 2015; 62: 1037–1046.

8. Schnell G, Tripathi R, Krishnan P, et al. Resistance characterization of hepatitis C virus genotype 2 from Japanese patients treated with ombitasvir and paritaprevir/ritonavir. *J Med Virol* 2018; 90: 109–119.

9. Okamoto H, Sugiyama Y, Okada S, et al. Typing hepatitis C virus by polymerase chain reaction with type-specific primers: application to clinical surveys and tracing infectious sources. *J Gen Virol* 1992; 73: 673–9.

10. Midgard H, Weir A, Palmateer N, et al. HCV epidemiology in high-risk groups and the risk of reinfection. *J Hepatol* 2016; 65: S33–45.

11. Vandelli C, Renzo F, Romanò L, et al. Lack of evidence of sexual transmission of hepatitis C among monogamous couples: results of a 10-year prospective follow-up study. *Am J Gastroenterol* 2004; 99: 855–859.

12. Terrault NA, Dodge JL, Murphy EL, et al. Sexual transmission of hepatitis C virus among monogamous heterosexual couples: the HCVpartners study. *Hepatology* 2013; 57: 881–889.

13. Thomas DL, Thio CL, Martin MP, et al. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. *Nature* 2009; 461: 798-801.

14. Sarrazin C, Isakov V, Svarovskaia ES, et al. Late relapse versus hepatitis C virus reinfection in patients with sustained virologic response after sofosbuvir-based therapies. *Clin Infect Dis* 2017; 64: 44–52.

15. Iio E, Shimada N, Takaguchi K, et al. Clinical evaluation of sofosbuvir/ledipasvir in patients with chronic hepatitis C genotype 1 with and without prior daclatasvir/asunaprevir therapy. *Hepatol Res* 2017; 47: 1308–1316.

Table. Clinical data at baseline and treatment outcome

	Case1	Case 2
Age (years)	55	60
Gender	male	female
Cirrhosis/non-cirrhosis	non-cirrhosis	non-cirrhosis
Liver stiffness (kPa)	9.9	13.3
Past IFN based therapy	-	-
WBC (/μL)	5700	3200
Hb (g/dL)	15.2	13.7
Plt ($\times 10^4$/μL)	18.9	12.6
T-Bil (mg/dL)	0.5	0.6
ALT (IU/L)	87	220
FIB-4 index	2.06	4.40
AFP (ng/mL)	5.8	47.2
HCV RNA viral load ($\log_{10}$ IU/mL)	5.6	5.7
Serotype	1	2
Genotype	1b	2b
Interleukin 28B rs8099917 SNP	TT	TT
DAA regimen	OMV+PTV+r	SOF＋RBV
Outcome after DAA therapy	relapse	Suspected reinfection

IFN, interferon; WBC, white blood cell; Hb, hemoglobin; Plt, platelet; T-Bil, total bilirubin; ALT, alanine aminotransferase; FIB-4 index, Fibrosis-4 index; AFP, α-fetoprotein; HCV, hepatitis C virus; SNP, single nucleotide polymophism; DAA, direct-acting antiviral; OMV, ombitasvir; PTV, paritaprevir; r, ritonavir; SOF, sofosbuvir; RBV, ribavirin; GLE, glecaprevir; PIB, pibrentasvir; SVR, sustained viral response.

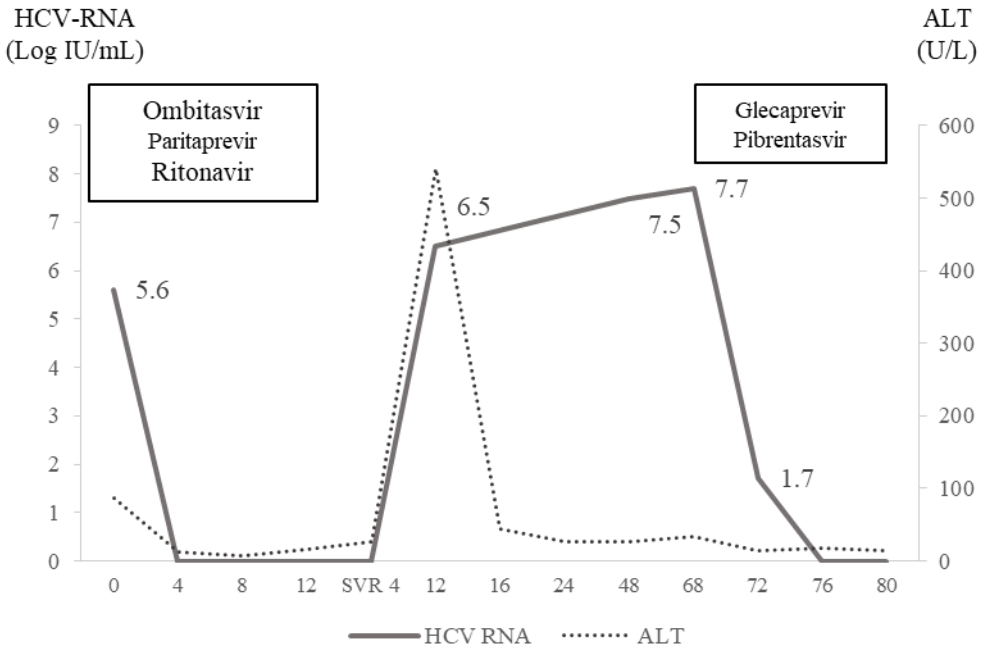
Figure Legends

Figure 1.

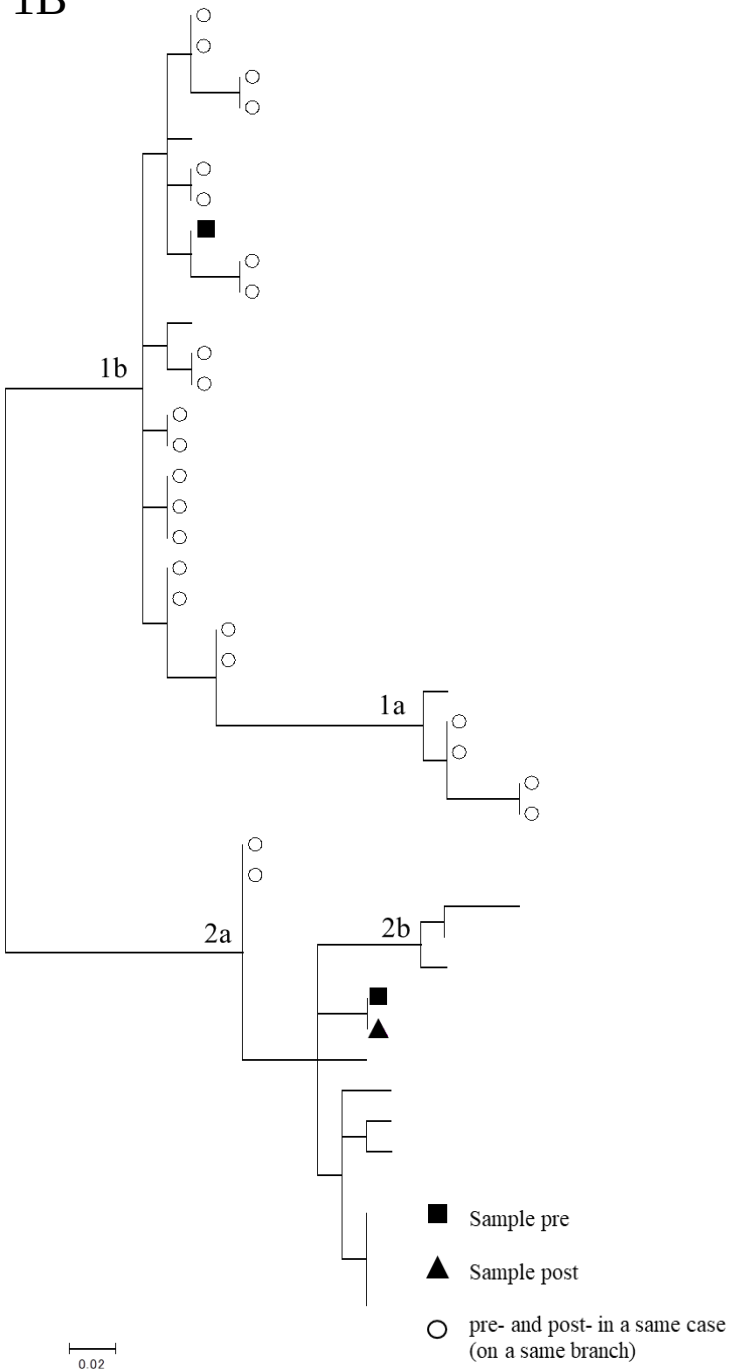
A. Clinical course of the serum HCV RNA and ALT levels in case 1.

B. Phylogenetic tree based on HCV NS5B sequences spanning 339 nucleotide positions (8,278-8,618) in case 1. Two strains were revealed at baseline: GT1b and GT2a. The black squares and triangle indicate the strains pre- and post- treatment, respectively. We showed different cases using white circles. These indicate a same patient at the pre-treatment and at the relapse.

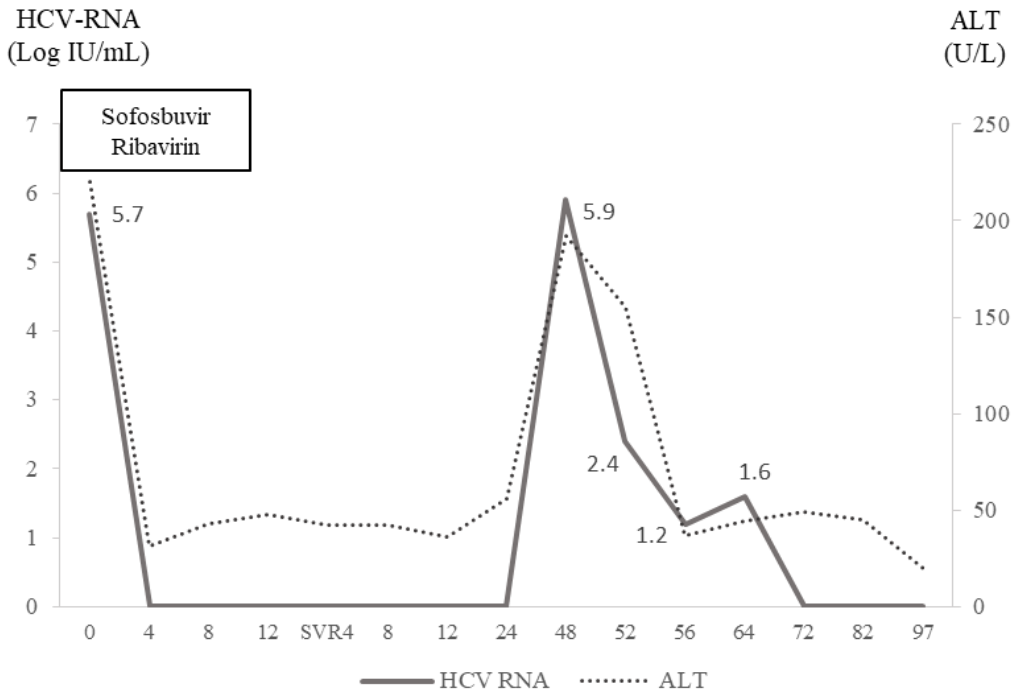
1A


HCV-RNA
(Log IU/mL)
ALT
(U/L)
Ombitasvir
Paritaprevir
Ritonavir
Glecaprevir
Pibrentasvir
5.6
6.5
7.5
7.7
1.7
0 4 8 12 SVR 4 12 16 24 48 68 72 76 80
HCV RNA
ALT

1B


1b
1a
2a
2b
0.02
Sample pre
Sample post
pre- and post- in a same case
(on a same branch)

2A


HCV-RNA
(Log IU/mL)
ALT
(U/L)
Sofosbuvir
Ribavirin
5.7
5.9
2.4
1.6
1.2
0
4
8
12
SVR4
8
12
24
48
52
56
64
72
82
97
HCV RNA
ALT

2B

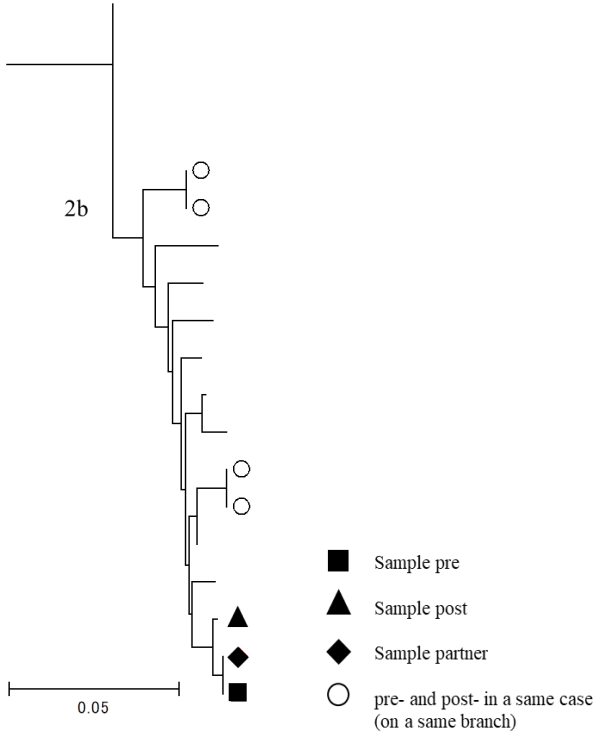

2b
0.05
Sample pre
Sample post
Sample partner
pre- and post- in a same case
(on a same branch)

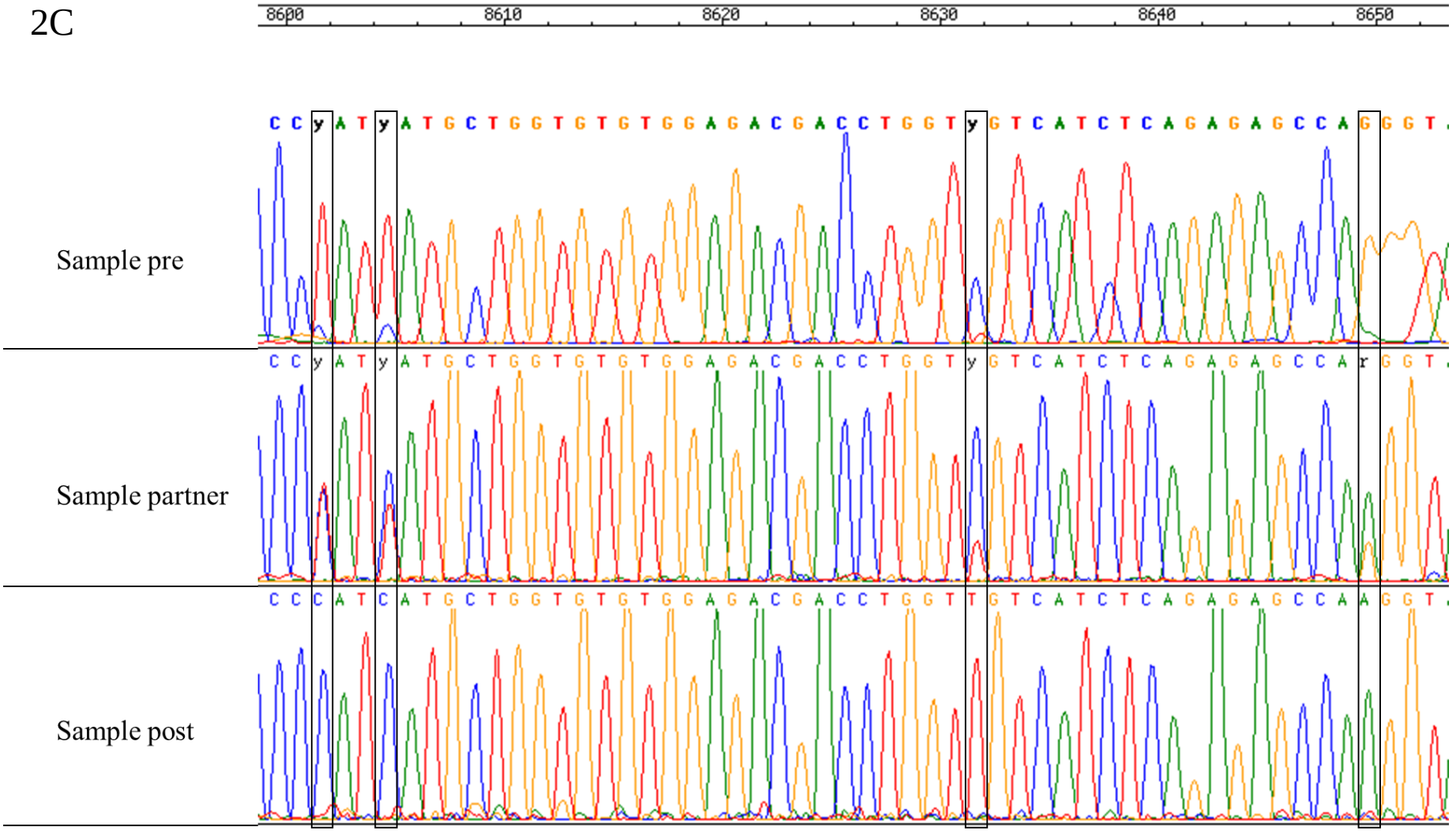
Figure 2.

A. Clinical course of the serum HCV RNA and ALT levels in case 2.

B. Phylogenetic tree analysis based on HCV NS5B sequences spanning 339 nt (nucleotide position: 8278-8618). Strains with pre and post HCV treatment obtained Case 2 patient were compared with those of other relapsed patients or reference sequences retrieved from the EMBL/DDBJ/GenBank database using MEGA6.0 software to analyze their evolutionary relationship.[15] White circles on a same branch indicate a same patient at the pre-treatment and at the relapse. Black square, triangle, and diamond indicated the strains with pre- and post-treatment of Case 2 and her partner, respectively. Phylogenetic analysis showed that those 3 strains had high homology.

C. Three Sequences indicated the strains with pre- and post- treatment of Case 2 and her partner, respectively.

2C


8600
8610
8620
8630
8640
8650
Sample pre
Sample partner
Sample post