Changes in Liver Steatosis as Well as Liver Fibrosis in Patients with Chronic Hepatitis C After Successful Direct-Acting Antiviral Therapy

Anahita Sadeghi[1], Roya Amiri[1], Elham Akbarpour[1], Babak Mirminachi[1], Amir-Houshang Sharifi[1], and Shahin Merat[1]

[1]Tehran University of Medical Sciences

September 11, 2020

Abstract

Introduction and Objectives: After successful treatment of hepatitis C virus (HCV) infection with direct-acting antivirals (DAAs), the stage of liver fibrosis decreases by time. Here, we aimed to assess the changes in liver fibrosis stage using transient elastography (TE) after successful DAA therapy in HCV-infected cirrhotic patients who referred to Shariati hospital from 2016 to 2017. Material and Methods: In this observational cohort, all HCV-infected cirrhotic patients who were treated with a combination of sofosbuvir/daclatasvir and had achieved sustained virologic response (SVR) and also had undergone pre- and post-treatment TE, were enrolled. The primary outcome was the changes in TE parameters six months after the end of treatment compared to baseline. Results: A total of 442 eligible subjects received DAA therapy. Overall, the SVR rate was 96.6%. Of these, 149 patients had completed the protocol and were enrolled. The mean age of patients was 56.1±10.3 years and the predominant sex was male (77.9%). The median (Q1-Q3) liver stiffness (LS) value at baseline was 26.3 kPa (18.1-38 kPa), which significantly decreased to 20.9 kPa (12-29.7 kPa) [z = -8.45, p-value < 0.001]. Also, the liver steatosis of patients with baseline CAP [?] 220 dB/m had a significant response to treatment [z = -2.3, p-value = 0.023]. Based on multivariate analysis, a higher baseline liver fibrosis stage was the only determinant of LS values improvement in our study. Conclusion: Successful HCV eradication in patients with liver fibrosis results in significant improvement in LS, even in cirrhotic patients.

Highlights

- Direct-acting antivirals both eradicate hepatitis C and induce reversal of liver fibrosis.
- The higher the fibrosis, the greater the regression.
- Eradication of hepatitis C virus may also decrease the hepatic steatosis.

Introduction

Hepatitis C virus (HCV) infection is a major global health problem, with approximately 170 million (2.3%) infected individuals worldwide.[1] These patients are more prone to develop chronic liver disease, cirrhosis and eventually hepatocellular carcinoma (HCC). HCV infection is recognized as an underlying cause in about a quarter of patients diagnosed with HCC and liver cirrhosis.[2-4] Based on statistical models, the prevalence of chronic HCV infection in Iran was estimated to be 186,500 in 2014 and is expected to increase to 213,700 by 2030.[5] Moreover, three-to-four-fold increase in the number of decompensated cirrhosis, HCC, and liver disease deaths because of HCV was speculated by 2030.[5]

It has been reported that about half of patients with chronic HCV infection demonstrate high grades of steatosis.[6]Severity of liver stiffness (LS) and fibrosis stage are of the most important prognostic factors in these patients. Antiviral therapy has shown to be effective in hepatic inflammation reduction and subsequent

fibrosis regression.[6] Also, a successful viral eradication with sustained virologic response (SVR) has been associated with decreased liver-related morbidity and mortality, including cirrhosis and HCC.[7,8] Consequently, a routine assessment of liver fibrosis and steatosis is recommended to be a part of management in chronic HCV-infected patients.

Interferon (IFN)-based therapy was routinely used for treatment of chronic HCV infection. Previous studies have reported that IFN-based therapies significantly reduce the alanine aminotransferase (ALT) level and severity of liver fibrosis. However, serious side effects have limited the application of this therapeutic regimen.[9-12] In recent years, appearance of direct-acting antiviral agents (DAAs) has made a great impact on the treatment of these patients with high SVR rate and favorable safety profile.[13] A fixed-dose combination of sofosbuvir 400 mg and daclatasvir 60 mg is the first DAA therapy that has become available in Iran. Although many studies have addressed the efficacy of this dual DAA therapy, there are still insufficient data on the effect of this regimen on liver fibrosis.[14,15]

Even though liver biopsy is being considered as a gold standard method for liver fibrosis evaluation, it is not routinely used during follow-up visits of chronic HCV-infected patients. Its invasiveness, possible sampling errors and diversity of the tests' interpretation in addition to the need for frequent liver evaluation lie among the reasons for its exclusion.[16] Therefore, it is essential to develop a feasible, non-invasive procedure. In this regard, transient elastography (TE) has been introduced as a valuable screening method for early detection of liver fibrosis in chronic liver diseases.[17-20] Besides, hepatic steatosis which is a major risk factor of HCC development in chronic HCV infection, could be measured quantitatively by controlled attenuation parameter (CAP) of TE.[21]

Here we aim to evaluate the changes in severity of liver fibrosis and steatosis using transient elastography method before and six months after the end of treatment (EOT) with the combination of sofosbuvir and daclatasvir in HCV-infected cirrhotic patients.

Material and Methods

This is a single-center prospective cohort study designed and performed in Hepatitis C clinic of Shariati hospital affiliated to Tehran University of Medical Sciences during 2016-2017. All HCV-infected subjects with cirrhosis (defined as initial LS greater than 12.5 KPa) were enrolled. Patients presenting with any of the following conditions were excluded: HIV co-infection, advanced renal failure (defined as eGFR $<$ 30ml/min/1.73m^2), organ transplant, HCC, and previous failure of NS5A inhibitor-containing regimen. The HCV diagnosis was confirmed by a sensitive polymerase chain reaction (PCR). A single once daily fixed-dose combination tablet containing 400 mg of sofosbuvir and 60 mg of daclatasvir (Sovodak, Fannavaran Rojan Mohaghegh Daru, Tehran, Iran) was administered either for 12 weeks with weight-based ribavirin (1000 or 1200 mg) or 24 weeks alone. TE was performed before and six months after end of treatment (EOT). Patients who could not undergo TE, did not achieve SVR, and lost to follow-up were excluded as well. The study protocol was in accordance with the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Institutional Review Board of the Digestive Disease Research Institute of Tehran University of Medical Science [IR.TUMS.DDRI.REC.1396.8] and written consent was obtained from all patients.

TE was performed using the FibroScan 502 machine (Echosens, Paris, France), by the same physician in all cases. Based on the manufacturer's guidelines, The M probe was used when the thoracic parameter was $<$ 75 cm, and the XL probe when the thoracic parameter was $>$ 75 cm. During procedure, the patient lied in a dorsal decubitus position and maximally abducted the right arm. The probe was positioned on the skin overlying the right lobe of the liver, through the intercostal spaces. The median value of at least 10 valid measurements was recorded for each subject and considered valid if the interquartile range was less than 30% of the estimated median. The results were expressed in Kilopascals. The same method was used for CAP measurement.

Statistical analysis:

Data were analyzed using SPSS software package version 26 (IBM, New York, USA). According to the type

of distribution estimated by one-sample Kolmogorov-Smirnov test, parametric or non-parametric analyses were performed. Results are reported as mean ± SD if normally distributed or median (Q_1-Q_3) otherwise. To compare pre- and post-treatment LS and CAP values, Wilcoxon signed-rank test was applied. Univariate analysis was performed by the Chi-square test ± the Fisher's exact test, the independent-samples T test, or the Mann-Whitney U test, as appropriate. A multiple regression analysis was used to predict the association between LS changes as a dependent variable and gender, age, body mass index (BMI), HCV viral load and genotype, baseline LS value, and history of receiving prior treatment as independent variables. A p-value below 0.05 was considered statistically significant.

Results

Patient characteristics:

A total of 149 HCV-infected cirrhotic patients (mean age of patients was 56.1±10.3 years, 77.9% male) meeting the inclusion and exclusion criteria were enrolled (Figure 1). Baseline patient characteristics are given in Table 1. Overall, 79 patients (53%) had no prior treatment for Hepatitis C (naïve), 51 patients (34.2%) had a history treatment failure with pegylated interferon alfa-2a with or without ribavirin, and the treatment history of 19 patients (12.8%) was not recorded. For treatment, 135 subjects (90.6%) received sofosbuvir 400mg, daclatasvir 60mg, and 1000 or 1200 mg of ribavirin for 12 weeks and 14 received (9.4%) only sofosbuvir and daclatasvir.

Post-treatment TE values:

LS improved in 124 patients (83.2%) six months after the EOT, but only 33.1% of them (41 patients) could achieve non-cirrhotic ranges (<12.5 kPa). The median (Q1-Q3) LS value at baseline was 26.3 KPa (12.6-75 KPa), which markedly decreased to 20.9 kPa (12-29.7 kPa) [z = -8.45, p-value < 0.001] (Figure 2).

The median (Q1-Q3) CAP value slightly fell from 237 dB/m (207-260 dB/m) to 229 dB/m (201-271 dB/m) within the same time frame, although not statistically significant [z = -0.34, p-value = 0.73]. Hence, we performed a sensitivity analysis on 79 patients with baseline CAP value greater than 220 dB/m. Considering this condition, the liver steatosis also had a significant response to treatment [z = -2.3, p-value = 0.023]. Table 2 summarizes the pre- and post-treatment values of LS. Based on univariate analysis, the only factor that had significant impact on LS improvement was baseline LS value [OR=-0.30, 95% CI (-1.88 - 5.03), p-value < 0.001]. This result remained significant after adjusting for potential confounding variables (gender, age, body mass index (BMI), HCV viral load, genotype, history of prior treatment) using multivariate regression analysis [OR=-0.28, 95% CI (-0.41 - -0.16), p-value < 0.001] (Table 3).

Discussion

In many liver diseases such as hepatitis B and autoimmune hepatitis, when the cause is effectively treated, regression of cirrhosis has been observed.[22-25] In the case of hepatitis C, in the interferon- era, several studies observed liver fibrosis regression in a significant number of HCV-infected cirrhotic patients (30-56%).[26,27] With the emergence of DAAs in recent years, there has been growing evidence of a similar effect of this pharmaceutical class. To our knowledge, this study is the first investigation in middle-east region in this regard.

Almost all studies comparing liver fibrosis before and after DAA treatment, have shown a significant decrease in fibrosis score, regardless of the baseline characteristics or treatment regimen. In a study by Tada et al, the effect of dual DAA therapy with daclatasvir and asunaprevir on liver stiffness of all HCV-infected patients who achieved SVR (both cirrhotic and non-cirrhotic), was assessed by shear wave elastography at baseline, EOT, and six months after the EOT and they reported a significant decrease in the post-treatment LS values.[28] Another Japanese study with the same regimen and a follow-up period of 72 weeks, indicated a significant, gradual and steady decline in LS values.[29] Another study from Egypt with sofosbuvir-based therapies also supported the same results.[30] Our findings are in agreement with previous studies too.

We evaluated the association of demographic factors, HCV characteristics, any prior treatment experience, as

well as, baseline liver fibrosis status with changes in LS in patients achieving SVR. Based on our results, higher baseline scores of liver fibrosis was independently associated with better improvement of LS values after DAA therapy. Comparing the results of previously published studies estimating the predictors of LS regression after DAA therapy, the advanced baseline liver fibrosis stage was the most pronounced factor.[28,31-33]Previous studies on Pegylated interferon and ribavirin demonstrated several factors to be significantly associated with improvement in LS values, such as baseline fibrosis stage, BMI, age, viral load, and genotype1.[34,35] However, our results as well as earlier data from Japanese patients indicated that the regression of liver fibrosis after DAA therapy is more probably influenced by baseline LS scores.[28] Additionally, low platelet, high serum angiopoietin-2, low ALT, diabetes, and esophageal varices were of other proposed factors to have an inverse association with significant LS reduction.[36-38] The effects of these predictive factors need to be further evaluated in larger populations with longer follow-up.

The severity of liver steatosis, represented as CAP value, improved in half of our patients. However, the improvement did not achieve statistical significance. The observed difference was meaningful when the analysis was limited to patients with CAP value [?] 220 dB/m, an optimal cut-off point associated with hepatic steatosis decline after HCV eradication suggested by a recently published study from Japan in which patients with higher baseline CAP value experienced significant decline six months after HCV eradication.[39] These findings are also supported by two other studies conducted in Japan.[40,41] The higher the baseline CAP value, the greater the therapeutic effect appears to be. As explained in a study by Powell et al, this is mostly because of "burn out" phenomenon, in which hepatic steatosis might already have been replaced by fibrosis.

It seems that lipids play a pivotal role in HCV life cycle.[42] That is why HCV infection might induce hepatic steatosis. Animal studies have explained that the HCV core protein inhibits the microsomal triglyceride transfer protein activity and very low density lipoprotein secretion in hepatocytes.[43] The other molecular mechanism underlying this phenomenon is that HCV upregulates the secretion of sterol-regulatory element binding proteins (SREBPs), resulting in increased uptake of lipoprotein particles by infected cells.[44] These mechanisms support our findings that HCV eradication could lessen the severity of hepatic steatosis. Considering the substantial amount of liver steatosis as an independent risk factor for HCC development, long-term careful monitoring of patients with post-eradication hepatic steatosis is crucial.[45,46]

Conclusions

To conclude, our results demonstrate that treatment with sofosbuvir/daclatasvir is highly effective in liver fibrosis regression in HCV-infected cirrhotic patients. Furthermore, HCV eradication in patients with higher levels of hepatic steatosis led to improvement in steatosis (CAP value). Future studies with larger sample size, longer follow-up period, and more precise methods for liver histology assessment are required to strengthen the current findings, overcome limitations, and elucidate all affective factors contributing to liver fibrosis regression in chronic HCV patients receiving DAAs therapy.

References

1. Disease GBD, Injury I, Prevalence C. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet.*2018;392(10159):1789-858.

2. Perz JF, Armstrong GL, Farrington LA, Hutin YJ, Bell BP. The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide. *J Hepatol.*2006;45(4):529-38.

3. Hepatitis C guidance: AASLD-IDSA recommendations for testing, managing, and treating adults infected with hepatitis C virus.*Hepatology.* 2015;62(3):932-54.

4. European Association for Study of L. EASL Recommendations on Treatment of Hepatitis C 2015. *J Hepatol.* 2015;63(1):199-236.

5. Hajarizadeh B, Razavi-Shearer D, Merat S, Alavian SM, Malekzadeh R, Razavi H. Liver Disease Burden

of Hepatitis C Virus Infection in Iran and the Potential Impact of Various Treatment Strategies on the Disease Burden. *Hepat Mon.* 2016;16(7):e37234-e.

6. Singh S, Fujii LL, Murad MH, Wang Z, Asrani SK, Ehman RL, et al. Liver stiffness is associated with risk of decompensation, liver cancer, and death in patients with chronic liver diseases: a systematic review and meta-analysis. *Clin Gastroenterol Hepatol.*2013;11(12):1573-84.e1-2; quiz e88-9.

7. Malekzadeh R, Mohamadnejad M, Rakhshani N, Nasseri-Moghaddam S, Merat S, Tavangar SM, et al. Reversibility of cirrhosis in chronic hepatitis B. *Clin Gastroenterol Hepatol.* 2004;2(4):344-7.

8. van der Meer AJ, Veldt BJ, Feld JJ, Wedemeyer H, Dufour JF, Lammert F, et al. Association between sustained virological response and all-cause mortality among patients with chronic hepatitis C and advanced hepatic fibrosis. *Jama.* 2012;308(24):2584-93.

9. Jabbari H, Zamani F, Hatami K, Sheikholeslami A, Fakharzadeh E, Shahzamani K, et al. Pegaferon in hepatitis C: Results of a Multicenter Study. *Middle East J Dig Dis.* 2011;3(2):110-4.

10. Jabbari H, Bayatian A, Sharifi AH, Zaer-Rezaee H, Fakharzadeh E, Asadi R, et al. Safety and efficacy of locally manufactured pegylated interferon in hepatitis C patients. *Arch Iran Med.*2010;13(4):306-12.

11. Yoshida H, Shiratori Y, Moriyama M, Arakawa Y, Ide T, Sata M, et al. Interferon therapy reduces the risk for hepatocellular carcinoma: national surveillance program of cirrhotic and noncirrhotic patients with chronic hepatitis C in Japan. IHIT Study Group. Inhibition of Hepatocarcinogenesis by Interferon Therapy. *Ann Intern Med.*1999;131(3):174-81.

12. Ikeda K, Saitoh S, Arase Y, Chayama K, Suzuki Y, Kobayashi M, et al. Effect of interferon therapy on hepatocellular carcinogenesis in patients with chronic hepatitis type C: A long-term observation study of 1,643 patients using statistical bias correction with proportional hazard analysis. *Hepatology.* 1999;29(4):1124-30.

13. Ferreira VL, Assis Jarek NA, Tonin FS, Borba HH, Wiens A, Pontarolo R. Safety of interferon-free therapies for chronic hepatitis C: a network meta-analysis. *J Clin Pharm Ther.* 2016;41(5):478-85.

14. Merat S, Sharifi AH, Haj-Sheykholeslami A, Poustchi H, Fattahi B, Nateghi-Baygi A, et al. The Efficacy of 12 Weeks of Sofosbuvir, Daclatasvir, and Ribavirin in Treating Hepatitis C Patients with Cirrhosis, Genotypes 1 and 3. *Hepat Mon.* 2017;17(1):e44564.

15. Merat S. SD1000: High Sustained Viral Response Rate in 1361 Patients With Hepatitis C Genotypes 1, 2, 3, and 4 Using a Low-cost, Fixed-dose Combination Tablet of Generic Sofosbuvir and Daclatasvir: A Multicenter, Phase III Clinical Trial. *Clin Infect Dis.* 2019.

16. Regev A, Berho M, Jeffers LJ, Milikowski C, Molina EG, Pyrsopoulos NT, et al. Sampling error and intraobserver variation in liver biopsy in patients with chronic HCV infection. *Am J Gastroenterol.*2002;97(10):2614-8.

17. Carrion JA, Navasa M, Bosch J, Bruguera M, Gilabert R, Forns X. Transient elastography for diagnosis of advanced fibrosis and portal hypertension in patients with hepatitis C recurrence after liver transplantation. *Liver Transpl.* 2006;12(12):1791-8.

18. Lemoine M, Katsahian S, Ziol M, Nahon P, Ganne-Carrie N, Kazemi F, et al. Liver stiffness measurement as a predictive tool of clinically significant portal hypertension in patients with compensated hepatitis C virus or alcohol-related cirrhosis. *Aliment Pharmacol Ther.*2008;28(9):1102-10.

19. Kim SU, Lee JH, Kim DY, Ahn SH, Jung KS, Choi EH, et al. Prediction of liver-related events using fibroscan in chronic hepatitis B patients showing advanced liver fibrosis. *PLoS One.* 2012;7(5):e36676.

20. Corpechot C, Carrat F, Poujol-Robert A, Gaouar F, Wendum D, Chazouilleres O, et al. Noninvasive elastography-based assessment of liver fibrosis progression and prognosis in primary biliary cirrhosis.*Hepatology.* 2012;56(1):198-208.

21. Ohata K, Hamasaki K, Toriyama K, Matsumoto K, Saeki A, Yanagi K, et al. Hepatic steatosis is a risk factor for hepatocellular carcinoma in patients with chronic hepatitis C virus infection. *Cancer.*2003;97(12):3036-43.

22. Malekzadeh R, Mohamadnejad M, Nasseri-Moghaddam S, Rakhshani N, Tavangar SM, Sohrabpour AA, et al. Reversibility of cirrhosis in autoimmune hepatitis. *Am J Med.* 2004;117(2):125-9.

23. Mohamadnejad M, Malekzadeh R, Nasseri-Moghaddam S, Hagh-Azali S, Rakhshani N, Tavangar SM, et al. Impact of immunosuppressive treatment on liver fibrosis in autoimmune hepatitis. *Dig Dis Sci.*2005;50(3):547-51.

24. Schiff ER, Lee SS, Chao YC, Kew Yoon S, Bessone F, Wu SS, et al. Long-term treatment with entecavir induces reversal of advanced fibrosis or cirrhosis in patients with chronic hepatitis B. *Clin Gastroenterol Hepatol.* 2011;9(3):274-6.

25. Marcellin P, Gane E, Buti M, Afdhal N, Sievert W, Jacobson IM, et al. Regression of cirrhosis during treatment with tenofovir disoproxil fumarate for chronic hepatitis B: a 5-year open-label follow-up study.*Lancet.* 2013;381(9865):468-75.

26. Maylin S, Martinot-Peignoux M, Moucari R, Boyer N, Ripault MP, Cazals-Hatem D, et al. Eradication of hepatitis C virus in patients successfully treated for chronic hepatitis C. *Gastroenterology.*2008;135(3):821-9.

27. Camma C, Di Bona D, Schepis F, Heathcote EJ, Zeuzem S, Pockros PJ, et al. Effect of peginterferon alfa-2a on liver histology in chronic hepatitis C: a meta-analysis of individual patient data.*Hepatology.* 2004;39(2):333-42.

28. Tada T, Kumada T, Toyoda H, Mizuno K, Sone Y, Kataoka S, et al. Improvement of liver stiffness in patients with hepatitis C virus infection who received direct-acting antiviral therapy and achieved sustained virological response. *J Gastroenterol Hepatol.*2017;32(12):1982-8.

29. Ogasawara N, Kobayashi M, Akuta N, Kominami Y, Fujiyama S, Kawamura Y, et al. Serial changes in liver stiffness and controlled attenuation parameter following direct-acting antiviral therapy against hepatitis C virus genotype 1b. *J Med Virol.* 2018;90(2):313-9.

30. Elsharkawy A, Alem SA, Fouad R, El Raziky M, El Akel W, Abdo M, et al. Changes in liver stiffness measurements and fibrosis scores following sofosbuvir based treatment regimens without interferon.*J Gastroenterol Hepatol.* 2017;32(9):1624-30.

31. Soliman H, Ziada D, Salama M, Hamisa M, Badawi R, Hawash N, et al. Predictors for Fibrosis Regression in Chronic HCV Patients after the Treatment with DAAS: Results of a Real-world Cohort Study. *Endocr Metab Immune Disord Drug Targets.* 2020;20(1):104-11.

32. Lledo GM, Carrasco I, Benitez-Gutierrez LM, Arias A, Royuela A, Requena S, et al. Regression of liver fibrosis after curing chronic hepatitis C with oral antivirals in patients with and without HIV coinfection. *Aids.* 2018;32(16):2347-52.

33. Tag-Adeen M, Sabra AM, Akazawa Y, Ohnita K, Nakao K. Impact of hepatitis C virus genotype-4 eradication following direct acting antivirals on liver stiffness measurement. *Hepat Med.*2017;9:45-53.

34. Poynard T, McHutchison J, Manns M, Trepo C, Lindsay K, Goodman Z, et al. Impact of pegylated interferon alfa-2b and ribavirin on liver fibrosis in patients with chronic hepatitis C. *Gastroenterology.*2002;122(5):1303-13.

35. Wang JH, Changchien CS, Hung CH, Tung WC, Kee KM, Chen CH, et al. Liver stiffness decrease after effective antiviral therapy in patients with chronic hepatitis C: Longitudinal study using FibroScan. *J Gastroenterol Hepatol.* 2010;25(5):964-9.

36. Knop V, Mauss S, Goeser T, Geier A, Zimmermann T, Herzer K, et al. Dynamics of liver stiffness by transient elastography in patients with chronic hepatitis C virus infection receiving direct-acting antiviral

therapy-Results from the German Hepatitis C-Registry. *J Viral Hepat.* 2020.

37. Kawagishi N, Suda G, Kimura M, Maehara O, Shimazaki T, Yamada R, et al. High serum angiopoietin-2 level predicts non-regression of liver stiffness measurement-based liver fibrosis stage after direct-acting antiviral therapy for hepatitis C. *Hepatol Res.* 2020.

38. Persico M, Rosato V, Aglitti A, Precone D, Corrado M, De Luna A, et al. Sustained virological response by direct antiviral agents in HCV leads to an early and significant improvement of liver fibrosis. *Antivir Ther.* 2018;23(2):129-38.

39. Kawagishi N, Suda G, Nakamura A, Kimura M, Maehara O, Suzuki K, et al. Liver steatosis and dyslipidemia after HCV eradication by direct acting antiviral agents are synergistic risks of atherosclerosis. *PLoS One.* 2018;13(12):e0209615.

40. Tada T, Kumada T, Toyoda H, Sone Y, Takeshima K, Ogawa S, et al. Viral eradication reduces both liver stiffness and steatosis in patients with chronic hepatitis C virus infection who received direct-acting anti-viral therapy. *Aliment Pharmacol Ther.* 2018;47(7):1012-22.

41. Shimizu K, Soroida Y, Sato M, Hikita H, Kobayashi T, Endo M, et al. Eradication of hepatitis C virus is associated with the attenuation of steatosis as evaluated using a controlled attenuation parameter. *Sci Rep.* 2018;8(1):7845.

42. Miyanari Y, Atsuzawa K, Usuda N, Watashi K, Hishiki T, Zayas M, et al. The lipid droplet is an important organelle for hepatitis C virus production. *Nat Cell Biol.* 2007;9(9):1089-97.

43. Perlemuter G, Sabile A, Letteron P, Vona G, Topilco A, Chretien Y, et al. Hepatitis C virus core protein inhibits microsomal triglyceride transfer protein activity and very low density lipoprotein secretion: a model of viral-related steatosis. *Faseb j.* 2002;16(2):185-94.

44. Syed GH, Tang H, Khan M, Hassanein T, Liu J, Siddiqui A. Hepatitis C virus stimulates low-density lipoprotein receptor expression to facilitate viral propagation. *J Virol.* 2014;88(5):2519-29.

45. Kurosaki M, Hosokawa T, Matsunaga K, Hirayama I, Tanaka T, Sato M, et al. Hepatic steatosis in chronic hepatitis C is a significant risk factor for developing hepatocellular carcinoma independent of age, sex, obesity, fibrosis stage and response to interferon therapy. *Hepatol Res.* 2010;40(9):870-7.

46. Tanaka A, Uegaki S, Kurihara H, Aida K, Mikami M, Nagashima I, et al. Hepatic steatosis as a possible risk factor for the development of hepatocellular carcinoma after eradication of hepatitis C virus with antiviral therapy in patients with chronic hepatitis C. *World J Gastroenterol.* 2007;13(39):5180-7.

Hosted file

`Figure 1.docx` available at https://authorea.com/users/357320/articles/479905-changes-in-liver-steatosis-as-well-as-liver-fibrosis-in-patients-with-chronic-hepatitis-c-after-successful-direct-acting-antiviral-therapy

Hosted file

`Figure 2.docx` available at https://authorea.com/users/357320/articles/479905-changes-in-liver-steatosis-as-well-as-liver-fibrosis-in-patients-with-chronic-hepatitis-c-after-successful-direct-acting-antiviral-therapy

Hosted file

`Table 1.docx` available at https://authorea.com/users/357320/articles/479905-changes-in-liver-steatosis-as-well-as-liver-fibrosis-in-patients-with-chronic-hepatitis-c-after-successful-direct-acting-antiviral-therapy

Hosted file

Table 2.docx available at https://authorea.com/users/357320/articles/479905-changes-in-liver-steatosis-as-well-as-liver-fibrosis-in-patients-with-chronic-hepatitis-c-after-successful-direct-acting-antiviral-therapy

Hosted file

Tables 3.docx available at https://authorea.com/users/357320/articles/479905-changes-in-liver-steatosis-as-well-as-liver-fibrosis-in-patients-with-chronic-hepatitis-c-after-successful-direct-acting-antiviral-therapy