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Changes in Liver Stiffness and Steatosis in Patients with Chronic Hepatitis C during the Clinical Course of Daclatasvir/ Velpatasvir Therapy: A Prospective Observational Study

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Abstract

Background: The mechanism behind reduction of liver fibrosis and steatosis in Chronic Hepatitis C (CHC) patients receiving interferon-free Direct Acting Antiviral Agent (DAA) therapy is still unclear. Non-invasive techniques such as Transient Elastography (TE) and Controlled Attenuation Parameter (CAP) are used to evaluate the chronological changes in liver stiffness and steatosis, respectively, in CHC patients receiving DAA therapy. **Subjects and Methods:** The study involves 50 CHC patients receiving Daclatasvir or Velpatasvir (DCV/VEL) in whom liver stiffness and steatosis was measured using TE and CAP, respectively. Laboratory investigational data, liver stiffness, and steatosis were recorded at baseline (prior to therapy), week 12 (post initiation of therapy), End of Treatment (EOT), and week 24 (post initiation of therapy). Analysis was performed 12 weeks post completion of therapy (SVR 12). **Results:** Significant difference was observed in laboratory parameters such as aminotranferases, serum albumin, platelet count, and α-fetoprotein at each time point of study as compared to the baseline. A highly statistically significant difference was observed in laboratory parameters when calculated as overall (P<0.001). Liver stiffness reduced significantly at baseline vs week 12 (P=0.044), week 12 vs EOT (P=0.011), baseline vs week 24 (P<0.001) (overall P<0.001). However, steatosis showed an insignificant difference (overall P=0.325). **Conclusion:** A significant overall decrease was observed in liver stiffness among patients with chronic hepatitis C during the clinical course of Daclatasvir/ Velpatasvir therapy. Steatosis reduced considerably, however the results were found to be statistically insignificant.

Keywords: Chronic Hepatitis C; Liver Stiffness; Daclatasvir; Velpatasvir.

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Introduction

Approximately 180 million individuals worldwide are chronically infected with Hepatitis C Virus (HCV), a common cause of chronic liver disease and primary hepatocellular carcinoma.[1,2] Long term infection with HCV induces progressive liver fibrosis, resulting in development of cirrhosis and hepatocellular carcinoma.[3]

Interferon based therapy has been used to treat chronic HCV infection. Studies suggest that interferon based treatment is effective in reducing liver fibrosis, serum aminotransferase levels, and HCV RNA in Chronic Hepatitis C (CHC) patients by selective inhibition of HCV proteins like NS5A, nonstructural protein NS 3/4A protease, and NS5B polymerase.[4-8] However, it is associated with several treatment related adverse drug reactions (ADRs) such a fatigue, flu- like symptoms, and gastrointestinal disorders.

Interferon free Direct- Acting Antiviral agents (DAA) have recently been developed to treat CHC, as they result in increased Sustained Virological Response (SVR) rates, shorter and simpler treatment regimens, with minimal ADRs. Furthermore, treatment with DAA reduces liver stiffness in CHC patients, which in turn decreases the incidence of cirrhosis and hepatocellular carcinoma, causes regression of hepatitis activity and fibrosis, ameliorates hepatic inflammation, and improves long term quality of life. In India, the combination of Sofosbuvir with Velpatasvir (VEL) or Daclatasvir (DCV) was approved for use in the year 2017.

Although most HCV associated liver damage is immunomediated, some histopathological features, such as liver steatosis, suggest a cytopathic effect.[9-11] In patients with CHC, the prevalence of steatosis ranges from 40 to 86%.[12] Most patients have mild steatosis that affects less than 30% hepatocytes.

Assessment of the severity of liver fibrosis and steatosis is important when evaluating the stage of CHC. Transient Elastography (TE) is a useful technique that allows for rapid and non-invasive measurement of tissue stiffness.[13] A

meta-analysis has shown that liver stiffness as measured by TE accurately reflects liver fibrosis.[14] In addition, recent interest has shifted toward the Controlled Attenuation Parameter (CAP), which is based on the properties of ultrasound signals acquired using TE.[15] It has been reported that CAP can evaluate liver steatosis non-invasively in patients with chronic liver disease.[16]

After extensive Medline research, limited literature was found regarding the evaluation of changes in liver stiffness and steatosis in CHC patients during the clinical course of DCV/VEL therapy.

In this study we evaluated the chronological changes in liver stiffness and steatosis based on Transient Elastography and Controlled Attenuation Parameter in patients with chronic hepatitis C receiving Daclatasvir/ Velpatasvir therapy. Specifically, we analysed laboratory data, liver stiffness, and steatosis up to 12 weeks after the end of treatment (EOT).

Subjects and Methods

After obtaining written informed consent from patients, this prospective, observational study was conducted in Department of Gastroenterology, Teerthankar Mahaveer Medical College and Research Centre, Moradabad, from January to August 2018. The study enrolled 50 CHC patients with Genotype-3, aged between 20-70 years, receiving DCV/VEL therapy. Patients with co-morbidities such as Hepatocellular Carcinoma, HbsAG/ HIV Infection, Chronic Liver Disease, Chronic Alcoholic Disease, Severe Fatty Liver, Diabetes, Autoimmune Hepatitis, Primary Biliary Cirrhosis, Hemochromatosis, Wilson's disease, history of Congestive Heart Failure, alcohol consumption> 80 g/day, and those receiving hepatotoxic drugs were excluded from the study. Demographic data of all subjects was collected at during enrollment which included age, weight, height, body mass index (BMI).

At baseline, HCV infection was confirmed by both positive serum HCV antibody titers (ARCHITECT Anti-HCV; Abbott Laboratories, Abbott Park, Illinois, USA) and serum HCV RNA using a real- time PCR- based method (COBAS AmpliPrep/ COBAS TaqMan HCV Test; Roche Molecular Systems, Pleasanton, California, USA). Patients received a fixed dose combination of DCV (60 mg once daily) or VEL (100 mg once daily) with Sofosbuvir (400 mg once daily). The treatment regimen was scheduled for 12 weeks. Patients were asked to visit the clinic for the monitoring of treatment effects and adverse effects every 2 weeks throughout the treatment period. Liver stiffness, steatosis, laboratory data, and serum HCV RNA levels were measured at baseline, week 12 (post initiation of therapy), EOT (end of treatment), and week 24 (post initiation of therapy).

Liver stiffness was measured with TE (M-probe, Fibroscan; EchoSens, Paris, France). TE was performed after a 12 hour fast. The TE system generates a 50-Hz shear wave that is longitudinally polarized along the ultrasound axis. The tip of the transducer probe was placed on the surface of the skin surface between the ribs over the right lobe of the liver. The median value from 10 measurements performed at depths ranging from 25 to 65mm was used as the final liver stiffness value, which was expressed in kilopascals (kPa). The median value was considered to represent the elastic modulus of the liver. The criteria for reliability were 10 validated measurements and a success rate of at least 60%.[17] At the same time, liver steatosis was assessed using the CAP value provided by the device only when the liver stiffness measurements based on the same signals were valid, ensuring that liver ultrasound attenuation was obtained from the same volume of liver parenchyma as the liver stiffness measurement. CAP values were expressed in decibel-milliwatt (dB/m). Regarding steatosis, the presence of fatty liver (steatosis affecting ≥ 5% of hepatocytes) was defined as CAP values of at least 236 dB/m, based on a previous report.[18] One sinologist performed the TE examinations, who was blinded to the patient's clinical data. Statistical analysis was performed using SPSS (version 20.0; SPSS Inc, Chicago, IL). The data is represented as median (interquartile range). Friedman test and Wilcoxon signed rank test using adjustment with Holm's method were used to analyze differences among continuous variables at baseline, week 12, EOT, and week 24. The value of $P < 0.05$ was accepted as statistically significant.

Results

Significant results were obtained on comparing baseline aminotranferases level, platelet count, and α-fetoprotein value with that at week 12 ($P<0.05$). Median liver stiffness at baseline and week 12 was found to be 8.4 (5.0–14.8) kPa & 5.8 (4.1–14.1) kPa, respectively ($P=0.044$). A comparable difference was observed in steatosis at baseline vs week 12 ($P=0.350$). [Table 1, 2]

Table 1: Patient characteristics.

Factor	Baseline (n=50)	Week 12 (n=50)	EOT (n=50)	Week 24 (n=49)
Age (years)	63.0 (52.0–74.0)	--	--	--
BMI (kg/m2)	21.7 (19.9–23.3)	--	--	--
AST (IU/L)	39 (24–54)	23 (18–27)	21 (17–26)	23 (18–27)
ALT (IU/L)	34 (21–53)	15 (13–24)	15 (11–18)	16 (12–23)
Sr. Alb. (g/dl)	4.0 (3.5–4.3)	4.0 (3.6–4.2)	4.1 (3.8–4.3)	4.2 (3.8–4.4)
T. Bil. (mg/dl)	0.7 (0.5–0.9)	0.7 (0.6–0.9)	0.7 (0.5–1.0)	0.7 (0.6–1.0)
Plt (×104/mm3)	15.1 (11.1–19.7)	15.9 (11.7–21.7)	17.0 (11.8–21.1)	17.5 (11.7–21.2)
AFP (ng/ml)	4.0 (2.7–7.1)	3.6 (2.4–5.2)	3.3 (2.2–4.8)	3.3 (2.5–4.3)

TE (kPa)	8.4 (5.0–14.8)	5.8 (4.1–14.1)	5.5 (4.1–11.8)	5.4 (4.0–13.4)
CAP (dB/m)	197 (159–231)	204 (182–224)	203 (175–225	210 (184–231)

Data represented as median (interquartile range). BMI= Body Mass Index; AST= Aspartate Aminotransferase; ALT= Alanine Aminotransferase; Sr. Alb.= Serum Albumin; T. Bil.= Total Bilirubin; Plt= Platelet Count; AFP= α-fetoprotein.

Table 2: P values for changes in continuous clinical data during and following DCV/ VEL therapy.

Factor	Baseline Vs Week 12a	Week 12 Vs EOTa	EOT Vs Week 24a	Baseline Vs Week 24a	Overall[b]
AST (IU/L)	<0.001*	0.021*	0.336	<0.001*	<0.001*
ALT (IU/L)	<0.001*	0.015*	0.622	<0.001*	<0.001*
Sr. Alb. (g/dl)	0.338	0.083	0.083	0.005*	<0.001*
T. Bil. (mg/dl)	1.000	1.000	1.000	1.000	0.695
Plt (×104/mm3)	0.002*	0.870	0.708	<0.001*	<0.001*
AFP (ng/ml)	0.007*	<0.001*	0.934	0.003*	<0.001*
TE (kPa)	0.044*	0.011*	0.054	<0.001*	<0.001*
CAP (dB/m)	0.350	1.000	1.000	0.350	0.325

BMI= Body Mass Index; AST= Aspartate Aminotransferase; ALT=Alanine Aminotransferase; Sr. Alb.= Serum Albumin; T. Bil.= Total Bilirubin; Plt= Platelet Count; AFP= α-fetoprotein.
aWilcoxon signed-rank test; bFriedman's test.
*Statistically significant difference between groups; (P < 0.05)

Aminotranferases level and α-fetoprotein value at week 12 vs EOT revealed significant difference (P<0.05). Median liver stiffness value at EOT was found to be 5.5 (4.1–11.8) kPa (Pat week 12 vs EOT= 0.011). However, steatosis revealed comparable results at week 12 vs EOT (P=1.000). [Table 1, 2]

Comparable results were obtained for various clinical parameters, liver stiffness, and steatosis at EOT vs week 24 (P>0.05). [Table 2]

Aminotranferases level, serum albumin, platelet count, and α-fetoprotein value revealed significant results at baseline vs week 24 (P<0.05). 5.4 (4.0–13.4) kPa was recorded as the median liver stiffness value at week 24 (P at baseline vs week 24 <0.001). A comparable difference was observed in steatosis at baseline vs week 24 (P=0.350). [Table 1, 2]

Furthermore, an overall intergroup comparison for various clinical parameters revealed highly statistically significant results (P<0.001). Liver stiffness showed significant, whereas steatosis revealed comparable results in the overall intergroup comparison (P<0.001, P=0.325, respectively). [Table 2]

Discussion

One of these fifty patients was excluded from analysis as he required treatment for hepatocellular carcinoma. As a result, final analysis included 49 patients.

In this study, liver stiffness decreased overall, with significant differences between baseline & week 12, week 12 & EOT, and baseline & week 24 in patients with chronic HCV infection who received DAA therapy. Liver stiffness is influenced not only by the degree of liver fibrosis but also by necro-inflammatory activity.[19,20]

Elevated aminotranferases levels, corresponding to the presence of necro-inflammatory activity, significantly decreased overall, as well as in between baseline & week 12, week 12 & EOT, and baseline & week 24. The reduction of aminotranferases levels from baseline to SVR12 was strongly associated with the reduction in liver stiffness as shown by TE. Therefore, it was considered that liver stiffness at SVR12 reflects the liver fibrosis in patients with HCV eradication as a result of DAA therapy.

TE was developed as a non-invasive modality for diagnosing liver fibrosis. Sporea et al. reported that a TE cut-off value of 6.8 kPa can differentiate between significant fibrosis (METAVIR F≥ 2) and absent or mild fibrosis with a positive predictive value of 98%, negative predictive value of 30.1%, sensitivity of 59.6%, specificity of 93.3%, and diagnostic performance (area under the receiver operating characteristics curve) of 0.77 in patients with chronic HCV infection.[21,22] Recently, Sporea et al. reported that mean liver stiffness values evaluated by TE were significantly lower at EOT and SVR12 than at baseline in patients with chronic HCV with cirrhosis who underwent non-interferon-based therapy for HCV. They found that approximately 60% of patients had lower liver stiffness values at EOT compared with baseline, whereas at SVR12, approximately 75% of patients had lower liver stiffness values than at baseline.[23] We also found that liver stiffness levels evaluated by TE improved overall in patients with chronic HCV infection who received DAA therapy.

Numerous extra hepatic manifestations of HCV infection have been reported. Cacoub et al. found that almost 40% of patients with HCV developed at least one extra hepatic manifestation during the course of disease.[24] Type 2 diabetes and atherosclerosis, two well-known major complications of the metabolic syndrome, are noteworthy, because HCV infection has been linked to their pathogenesis.[25]

In addition, chronic HCV infection is associated with steatosis as evidenced by the strong association between steatosis and HCV genotype 3 infection.[10,26] Patients with this genotype have an approximately five-fold higher probability of having moderate-to-severe steatosis than patients with non-genotype 3 infection, hinting that some viral sequences are responsible for fat accumulation.[27] Imajo et al. reported that the areas under the receiver operating characteristics curve for CAP were 0.88 for the diagnosis of grade≥ 1 steatosis (5–33% of hepatocytes with fat), 0.73 for the diagnosis of grade≥2 steatosis (33–66% of hepatocytes with fat), and 0.70 for the diagnosis of grade 3 steatosis (>66% of hepatocytes with fat) in patients with

non-alcoholic fatty liver disease. They also found that when the CAP cut-off value was 236dB/m, the sensitivity, specificity, positive predictive value, and negative predictive value for the diagnosis of grade≥ 1 steatosis were 82.3, 91.0, 98.8, and 66.7%, respectively.[18] However, in our study, CAP values showed a comparable difference at all recorded time intervals. Therefore, it was considered that liver steatosis reduces in patients with HCV eradication as a result of DAA therapy. Additional studies with long term follow-up periods should be performed in the future.

The limitations of this study were that the sample size was small and that the data were representative of only a single institution covering a limited region. Future directions include expanding the study by increasing the number of patients from other institutions and region.

Conclusion

Liver stiffness evaluated using TE significantly decreased overall in patients with chronic HCV infection who received DAA therapy. In particular, liver stiffness did not change significantly between EOT and week 24, a period corresponding to low necro-inflammatory activity based on low ALT levels. Therefore, liver stiffness at SVR12 might reflect liver fibrosis in the patients who received DAA therapy and achieved SVR. In addition, liver steatosis evaluated using CAP showed insignificant reduction from baseline to SVR12 in patients with chronic HCV infection and fatty liver who received DAA therapy.

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