REVISTA DO INSTITUTO DE MEDICINA TROPICAL DE SÃO PAULO

JOURNAL OF THE SÃO PAULO INSTITUTE OF TROPICAL MEDICINE

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Received: 30 August 2020

Accepted: 14 December 2020

ORIGINAL ARTICLE

http://doi.org/10.1590/S1678-9946202163006

A real-life study on the impact of direct-acting antivirals in the treatment of chronic hepatitis C in liver transplant recipients at two university centers in Northeastern Brazil

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ABSTRACT

The efficacy of direct-acting antivirals (DAAs) in the treatment of chronic hepatitis C (CHC) in liver transplant recipients is poorly understood, and several factors, including immunosuppression, drug interactions, elevated viraemia, and intolerance to ribavirin (RBV), can reduce cure rates. We conducted a real-life study on liver transplant recipients with CHC treated with a combination of sofosbuvir (SOF) and daclatasvir (DCV) or simeprevir (SIM), with or without RBV, followed-up for 12 to 24 weeks. The treatment effectiveness was assessed by determining the sustained virological response (SVR) rates at 12 or 24 weeks after the treatment cessation. Eighty-four patients were evaluated, with a mean age of 63.4 ± 7.4 years, HCV genotype 1 being the most prevalent (63.1%). Nineteen patients (22.7%) had mild fibrosis (METAVIR < F2) and 41 (48.8%) significant fibrosis (METAVIR ≥ F2). The average time between liver transplantation and the start of treatment was 4 years (2.1-6.6 years). The SOF + DCV regimen was used in 58 patients (69%). RBV in combination with DAAs was used in seven patients (8.3%). SVR was achieved in 82 patients (97.6%), and few relevant adverse events could be attributed to DAA therapy, including a patient who stopped treatment due to a headache. There was a significant reduction in ALT, AST, GGT and FA levels, or the APRI index after 4 weeks of treatment, which remained until 12/24 weeks post-treatment. DAA treatment of CHC in liver-transplanted patients achieved a high SVR rate and resulted in the normalization of serum levels of liver enzymes.

KEYWORDS: Chronic hepatitis C. Direct-acting antiviral. Liver transplant. Sofosbuvir. Effectiveness. Brazil. Liver transplant recipient.

INTRODUCTION

The global prevalence of hepatitis C virus (HCV) infection is estimated at 1%, corresponding to 71.1 million viraemic subjects worldwide[1,2]. Chronic hepatitis C (CHC) was the third leading cause of liver transplantation in the United States of America (USA) in 2017, and over the last 15 years, it has accounted for 54% of indications for liver transplantation due to cirrhosis in Europe[2]. In Brazil, in 2019, it has been estimated that 22,747 thousand people were infected with HCV[3], with genotypes 1 and 3 being the most frequent[4], and these genotypes are also predominant in Northeast Brazil[5]. Between 2000 and 2015, CHC accounted for more than 70% of the 46,314 deaths caused by viral hepatitis in Brazil[6].

A sustained virological response (SVR) is defined as undetectable HCV at 12 or more weeks after the end of treatment, which is associated with a reduction in serum levels of liver enzymes due to an improvement of the necro-inflammatory activity in the liver parenchyma, with subsequent reductions in the progression of liver fibrosis and mortality[7]. In patients with active CHC undergoing liver transplantation, recurrence of the infection in the transplanted liver is universal, with a negative impact on graft and patients' survival[3,7]. In these patients, the viral load may be even higher than in non-transplanted patients[7]. Due to the high rate of graft reinfection and accelerated progression of the liver disease, liver fibrosis is observed in approximately 30% to 40% of transplanted patients with CHC, and progression to cirrhosis occurs within approximately five years[8]. Accordingly, recipients' survival after liver transplantation are reduced in HCV-infected recipients compared to uninfected ones[7].

For many years, interferon (IFN)-based antiviral therapies have been the main treatment option for CHC[7]. The first generation of direct acting antivirals (DAAs), boceprevir and telaprevir, has been used in combination with pegylated (PEG)-IFN and ribavirin (RBV)[9]. However, these therapeutic regimens have limited efficacy, and cause several side effects, in addition to achieving only low SVR rates in transplant recipients[10]. The first studies employing second-generation DAAs, such as sofosbuvir, a nucleotide analogue that inhibits the HCV polymerase, in combination with simeprevir, a protease inhibitor, or daclatasvir, an NS5A inhibitor, in the treatment of CHC in liver transplant recipients, revealed SVR rates of approximately 80%, which were still lower than the SVR rates observed in non-transplanted patients that exceeds 90%[11,12].

As of 2015, the Brazilian Ministry of Health (MS) made CHC therapy available to patients in the Unified Health System (SUS), including sofosbuvir, combined with simeprevir or daclatasvir, with or without RBV[13]. Indication of treatment for transplant recipients was considered a priority, regardless of the stage of fibrosis[13,14]. Although DAAs have revolutionized the CHC therapy, some factors can reduce SVR rates in liver transplant recipients, including the presence of comorbidities, drug interactions and lower tolerance to RBV[7,14]. Based on previously published literature, there are reports on the treatment of HCV infections with DAAs in Brazil, reaching SVR rates between 92% and 95%[15-18]. However, only three studies included exclusively liver transplantrecipients, all in the South and Southeast regions of the country[19-21]. This study aimed to describe the SVR rates after treatment of patients with CHC having undergone liver transplantation and treatment with sofosbuvir and daclatasvir or simeprevir, with or without RBV, in a real life study in the Brazilian Northeast region.

MATERIALS AND METHODS

Study design and population

As part of this retrospective observational study, we included liver transplant recipients diagnosed with CHC, who had been treated with sofosbuvir and daclatasvir or simeprevir, with or without RBV. Patients were selected in two hepatology reference centers located in Northeast Brazil, from December 2015 to March 2019. For the recruitment, liver transplant recipients had to be aged 18 years or older, undergone liver transplantation, diagnosed with CHC based on the presence of anti-HCV antibodies for more than six months, present with positive HCV RNA detected by quantitative PCR, and subjected to treatment with second-generation DAAs. Pregnant women and those subjects coinfected with the human immunodeficiency virus (HIV) or who received other organ transplants were excluded from the study. Patients were followed-up until the assessment of SVR rates at 12 or 24 weeks after the end of treatment. During this period, interviews were conducted, and data were obtained from medical records using a standardized questionnaire for research purposes, which included demographic data and clinical characteristics. The variables studied were age, sex, viral load, HCV genotype, liver fibrosis staging (determined by liver biopsy or APRI and/or FIB4 scores), treatment status (naive or treated), immunosuppressants in use, therapeutic regimen and duration and response to treatment with DAAs. All clinical data of the patients were recorded in a research database. All individuals used immunosuppressive therapy: tacrolimus and/or mycophenolate sodium or mofetil and/or prednisone and/or sirolimus.

This study was approved by the Research Ethics Committee of the Health Sciences Center of the Federal University of Pernambuco (CCS-UFPE), Recife, Brazil, under the protocol Nº 2,383,189/2018. All patients who agreed to participate in the study signed a free and informed consent form (ICF).

Clinical and laboratory evaluations

Clinical evaluations and blood sampling for laboratory tests were carried out in the following periods: up to 3 months before the start of treatment, at week 4 of treatment, at the end of treatment, and 12 or 24 weeks after the end of treatment.Biochemical and hematological analyses included assessment of haemoglobin (Hb), platelets count, aspartate

aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), bilirubin (Bt), creatinine (Cr) and albumin levels. All analyses were performed in the laboratories of the two reference centers, and laboratory data were collected from medical records.

Quantification of HCV RNA

Quantification of HCV RNA was performed at the Central Laboratory of Pernambuco (LACEN-PE) using real-time PCR (qPCR) (Abbott Diagnostics®, Chigago, IL, USA), with a quantification range of 12 to 10^8 IU/mL, using the m2000 Real-Time System (Abbott®, Chicago, IL, USA)[22]. The 5' untranslated region (UTR) was used as a target for detection of HCV load[23].

HCV RNA was quantified up to 3 months before the start of treatment, at week 4 of treatment, at the end of treatment, and at week 12 or 24 after the end of treatment to assess the SVR rate. SVR was defined as undetectable levels of HCV RNA at 12 or 24 weeks after the end of the antiviral therapy. Virological failure was defined as detectable HCV RNA at 12 or 24 weeks after the end of treatment.

Assessment of hepatic fibrosis

The stage of liver fibrosis was assessed by biopsy performed up to one year before starting the treatment or by serological indices APRI (based on the AST ratio and platelets count), and FIB-4 (based on age, AST and ALT levels, and platelets count) up to three months before starting the treatment[24]. To assess the extent of liver fibrosis through biopsy, the METAVIR scoring system was used (F0 = no fibrosis; F1 = mild fibrosis; F2 = moderate fibrosis; F3 = advanced fibrosis; F4 = cirrhosis)[25]. For the APRI score, the lower and upper cutoff points of 0.5 and 1.50 were used to diagnose the absence and presence of significant fibrosis (METAVIR ≥ F2). For the FIB4 score, the lower and upper cut-off points of 1.45 and 3.35 were used to diagnose the absence and presence of advanced fibrosis (METAVIR ≥ F3)[26].

CHC therapy

The treatment scheme for CHC, including time, dosage, and whether or not RBV should be used, followed the Clinical Protocol and Therapeutic Guidelines for Hepatitis C and Coinfections (PCDT) 2015/2019[13,27], provided by the Brazilian Ministry of Health, which is based on the viral genotype, previous experimentation with DAAs, and fibrosis stage, summarised as follows:

- genotype 1: sofosbuvir plus simeprevir or daclatasvir, with or without RBV;
- genotype 2: sofosbuvir and RBV;
- genotype 3: sofosbuvir and daclatasvir, with or without RBV.

All treatment regimens were administered for 12 or 24 weeks. The addition of RBV was optional, especially when there were predictors of a worse virological response, such as cirrhosis or previous null response[13,27].

Statistical analysis

Regarding the descriptive analysis of the demographic and clinical data of the transplant recipients, the frequencies of qualitative variables, as well as the mean ± standard deviation or median (25^{th}-75^{th} percentile) of continuous variables were evaluated. The normal distribution of continuous variables was verified by applying the Shapiro-Wilk normality test.

Differences between the different time points of serial evaluations of biochemical and hematological parameters and hepatic fibrosis staging (before the start of treatment, at week 4 and at the end of treatment, and after 12 or 24 weeks after the end of treatment) were assessed using the paired t-test, whenever normality and homogeneity of variance testing allowed it, or the Wilcoxon signed-rank test for data with non-parametric distribution. Differences were considered statistically significant at a $p < 0.05$. The SPSS Statistics software, version 25 for Windows (IBM Corporation, Armonk, NY, USA) was used for statistical analyses.

RESULTS

Eighty-seven liver transplant recipients with CHC were evaluated between December 2015 and March 2019. Of these, three (3.4%) were excluded: one due to HIV coinfection, and two for having a transplanted kidney. The clinical and demographic characteristics of the 84 patients included in this study are shown in Table 1. Male sex predominated (63.1%), and the mean age was 63.4 ± 7.4 years. Genotype 1 was observed in 53 patients (63.1%). Diabetes mellitus and systemic arterial hypertension were the most frequent comorbidities, present in 48.9% and 52.3% of patients, respectively. Tacrolimus was used by 62 (74%) patients, either as single therapy or in combination with mycophenolate.

The median time between liver transplantation and start of the current treatment was 4 years (2.1-6.6 years). Among the 84 patients, 19 (22.7%) had mild fibrosis (METAVIR < F2), 41 (48.8%) had significant or moderate

Table 1 - Demographic and clinical characteristics of 84 liver transplant recipients with chronic hepatitis C, treated with sofosbuvir associated with daclatasvir or simeprevir, Northeast - Brazil, 2015-2019.

Characteristics	N = 84	
Gender, n (%)		
Male	53	(63.1)
Female	31	(36.9)
Age (years)*	63.4	(± 7.4)
Ethnicity, n (%)		
White	28	(33.3)
Mixed (black and white)	52	(61.9)
Black	4	(4.8)
State (Northeast – Brazil)		
Pernambuco	61	(72.6)
Paraiba	10	(11.9)
Alagoas	7	(8.3)
Others	6	(7.1)
Comorbidities	77	(91.7)
Diabetes mellitus	36	(42.9)
Systemic arterial hypertension	49	(58.3)
Hepatitis B	4	(4.8)
Obesity	3	(3.6)
HCV Genotype		
1	13	(15.4)
1 a	5	(6.0)
1 b	35	(41.7)
2	1	(1.2)
3	21	(25.0)
3 a	8	(9.5)
3 e 4	1	(1.2)
Immunosuppression		
Tacrolimus	21	(25.0)
Mycophenolate sodium	6	(7.1)
Tacrolimus + Mycophenolate sodium	38	(45.2)
Tacrolimus + mycophenolate mofetil	3	(3.6)
Prednisona + Mycophenolate sodium	5	(6.0)
Sirolimus + Mycophenolate sodium	11	(13.1)

†Mean ± standard deviation.

fibrosis (METAVIR = F2), and 24 (28.5%) had advanced liver fibrosis and/or cirrhosis (METAVIR > F2). Hepatocellular carcinoma (HCC) was diagnosed in five patients (6.0%) before CHC treatment; another two patients (2.4%) were diagnosed with HCC after the end of the current treatment. Both had undergone liver transplantation more than five years ago, one of them had mild fibrosis (METAVIR < F2), and the other had advanced liver fibrosis/ cirrhosis (METAVIR F3-F4).

Table 2 describes the treatment regimens and responses to DAAs. Among the 84 patients, 41 (48.8%) had previously been treated with IFN or PEG-INF together with RBV, as follows: 12 (14.3%) with INF + RBV and 20 (23.8%) with PEG + RBV. Two patients (2.4%) were treated with first-generation protease inhibitors, one with boceprevir and the other with telaprevir.

For the current treatment, combination therapy with SOF + DCV was the most frequent, which was used for treating 58 patients (69%), with RBV being administered in

Table 2 - Characteristics of previous and the current treatment of CHC with sofosbuvir associated with daclatasvir or simeprevir, in 84 liver transplant recipients with chronic hepatitis C, Northeast - Brazil, 2015-2019.

Characteristics	(N=84)	
Previous treatment, n (%)		
Yes	41	(48.8)
No information	1	(1.2)
Previous treatment regimen, n (%)		
Naive	42	(50.0)
IFN	3	(3.6)
IFN+RBV	12	(14.3)
PEG IFN	4	(4.8)
PEG IFN+RBV	20	(23.8)
Boceprevir	1	(1.2)
Telaprevir	1	(1.2)
No information	1	(1.2)
Previous treatment, n (%)		
Recurrent	7	(8.3)
Non-responder	25	(29.8)
Intolerant	9	(10.7)
No information	1	(1.2)
Current treatment regimen, n (%)		
SOF+SMV	19	(22.6)
SOF+DCV	58	(69.0)
SOF+DCV+RBV	6	(7.1)
SOF+SMV+RBV	1	(1.2)
Treatment duration, n (%)		
12 weeks	80	(95.2)
SVR, n (%)	82	(97.6)

†Mean ± standard deviation; IFN = interferon; Peg-IFN = egylated interferon; DCV = daclatasvir; SMV = simeprevir; SOF = sofosbuvir; RBV = ribavirin; SVR = sustained virological response.

six patients (7.1%). The SOF + SMV combination treatment was used in 19 patients (22.6%), with RBV administered to only one individual (1.2%). Among the 84 patients, SVR was achieved in 82 (97.6%). In 38 and 44 patients, HCV RNA screening was carried out 12 and 24 weeks after the end of treatment, respectively. The two patients for whom SVR was not achieved, did not complete the treatment with DAAs according to the proposed scheme, and were considered non-responders in the intention-to-treat assessment. One of them used the SOF + SIM combination treatment for only four weeks with errors, and another patient developed headache during the first week of SOF + SIM treatment and, on his own, suspended the therapeutic regimen.

Adverse events were recorded in 18 (25%) patients: fatigue (n = 6 - 8.3%), headache (n = 5 - 6.9%), abdominal pain in 4 (n = 4 - 5.6%), dizziness (n = 3 - 4.2%), nausea (n = 2 - 2.8%), diarrhea (n = 2- 2.8%), depressive symptoms (n = 1 - 1.4%), spontaneous sweating (n = 1 - 1.4%) and skin lesions (n = 1 - 1.4%) . In only one of these patients, the treatment was suspended due to a headache related to the use of DAAs. No drug interaction was observed with any of the antiviral regimens used. Prior to the prescription of the antiviral regimen, an assessment was made regarding possible interactions, particularly with the immunosuppressants in use.

Serial assessment of biochemical and hematological parameters, as well as liver fibrosis staging is described in Table 3 and Figure 1. Hemoglobin levels and platelet counts in transplant recipients remained stable at 12 and 24 weeks after the end of treatment. In addition, there was a significant reduction in the serum concentrations of ALT, AST, GGT and ALP at week 4, which was maintained up to 24 weeks after the treatment. The mean values of APRI and FIB-4 showed a significant reduction at week four of treatment, which was maintained until the end (Figure 2).

DISCUSSION

This real-life study describes the treatment of recurrent HCV infection after liver transplantation using DAAs in two public university hospitals in Northeast Brazil. It covers a specific population, for which the evolution of CHC is more aggressive, and whose treatment with IFN, even in the PEG formulation, presents a very low chance of SVR due to drug interactions, in addition to providing several side effects and difficulties in adjusting the doses of immunosuppressants.

This study included 84 liver transplant recipients with CHC, who were treated with second-generation DAAs, achieving a SVR of 97.6%. These data are similar to those observed in a French cohort, which evaluated treatment with SOF + CVD in 137 transplant patients with HCV recurrence, and showed a SVR rate of 96%, regardless of whether RBV was used[28]. Likewise, in the ALLY-1 study, which involved 53 liver transplant patients followed-up for 12 weeks and RVS12 was achieved in 95% and 91% of patients with genotypes 1 and 3, respectively[29]. The same was reported in a study by Gutierrez *et al.*[30], who evaluated treatment regimens of SOF + SMV for 12 weeks, and found SVR in 93.4% of 57 liver transplant recipients with CHC[30].

Three national studies have shown SVR rates between 90% and 98% after treatment of liver-transplanted patients with CHC using DAAs[19-21].The characteristics of the transplanted patients with CHC included in this study, i.e., associated predominantly with HCV genotype 1 and mild fibrosis, can justify the high SVR rates, very similar to those found by Araujo *et al.*[20] and Zanaga *et al.*[21]. In fact, in a study carried out in Sao Paulo including 53 patients, 23% of which had mild fibrosis (F0-F1), while 43% presented with moderate fibrosis (F2), the SVR rate was 98%, with virological failure occurring only in one cirrhotic patient[21]. On the other hand, a study by Mucenic *et al.*[19] used AEDs on 39 liver-transplanted patients with CHC in Southern Brazil, with a predominance of HCV genotype 3, revealing a SVR rate of 90%, which was slightly lower than that described in our study and the other two Brazilian studies[19]. It is possible that the lower response rate was due to the predominance of genotype 3 two thirds of the cases), and more patients with advanced fibrosis (F4) being included. These two factors, genotype 3 and advanced fibrosis/cirrhosis are predictive of lower SVR in both transplanted and non-transplanted patients[31,32].

When evaluating large samples, differences in SVR rates were observed according to the different genotypes. In a Brazilian study, including the largest survey in Latin America of patients with CHC treated with second-generation DAAs, coordinated by the Brazilian Society of Hepatology (BSH), including 20 Brazilian States and 3,939 patients, SVR rates were higher than 95% and about 90% in patients with genotypes 1 and 3, respectively, varying depending on the type of therapeutic scheme, sex, and the presence of decompensated cirrhosis[33]. The results presented in our study on transplanted patients with CHC are similar to those of the multicentre study of the BSH, possibly due to the very similar distribution of HCV genotypes, with a predominance of genotype 1[5,33]. Additionally, a multicentre study on the efficacy of DAAs upon treatment of 123 transplanted patients with CHC conducted in three transplant centres in the USA demonstrated that the SVR rate was lower in those with advanced fibrosis (METAVIR F3-F4, 81%) compared to those with mild and moderate fibrosis (METAVIR F0-F2, 93 %)[31]. Similar results were

Table 3 - Biochemical, hematological characteristics and liver fibrosis staging in 84 liver transplant recipients with chronic hepatitis C, treated with sofosbuvir associated with daclatasvir or simeprevir, Northeast - Brazil, 2015-2019.

Characteristics	Before treatment		Treatment week 4		End of Treatment		Week 12 post-treatment		Week 24 post-treatment		p1	p2	p3	p4
Hemoglobin (g/dL)†	13.2	±1.9	12.9	±2.3	12.9	±2.1	12.9	±1,2	14.1	±1.9	0.149[a]	0.552[a]	0.332[a]	**0.001[a]**
Platelets (x 10^3/mL)†	165	±68	174	±73	171	±63	178	±51	172	±74	0.300[a]	0.220[a]	0.133[a]	0.683[a]
AST (UI/L)‡	53.0	(34.0-85.0)	27.5	(20.0-34.5)	23.0	(17.0-31.0)	22.0	(17.0-31.2)	24.,0	(20.0-34.0)	**<0.001**[b]	**<0.001**[b]	**<0.001**[b]	**<0.001**[b]
ALT (UI/L)‡	70.0	(43.0-120.0)	26.5	(17.0-46.2)	20,0	(15.0-34.5)	22.5	(16.0-39.0)	24.0	(16.0-46.0)	**<0.001**[b]	**<0.001**[b]	**<0.001**[b]	**<0.001**[b]
GGT (UI/L)‡	137.5	(82.2-309.8)	85.5	(38.8-136.8)	62.0	(27.1-117.0)	75.6	(34.5-108.5)	63.0	(28,.5-122.5)	**<0.001**[b]	**<0.001**[b]	**0.002**[b]	**<0.001**[b]
ALP (UI/L)‡	119.0	(96.0-209.0)	99.0	(69.0-138.0)	92.0	(69,.0-138.0)	95.5	(61.8-173.8)	107	(75.0-151.0)	**<0.001**[b]	**<0.001**[b]	**<0.001**[b]	**<0.001**[b]
Total bilirrubin (mg/dL)‡	0.70	(0.50-1.00)	0.70	(0.50-0.88)	0.60	(0.40-0.83)	0.57	(0.39-0.90)	0.61	(0.46-0.87)	**0.040[b]**	**0.005**[b]	0.079[b]	**0.022[b]**
Albumin (mg/dL)‡	4.30	(4.00-4.59)	4.20	(3.92-4.40)	4.10	(4.00-4.40)	4,.20	(4.00-4.49)	4.25	(4.00-4.60)	0.055[b]	0.866[b]	0.810[b]	0.088[b]
Creatinine (mg/dL)†	1.03	±0.36	1.02	±0.32	1.08	±0.38	1.21	±0.46	1.03	±0.31	0.574[a]	0.326[a]	**0.016[a]**	0.072[a]
APRI‡	0.75	(0.52-1.68)	0.41	(0.29-0.69)	0.43	(0.27-0.76)	0.33	(0.25-0.69)	0.41	(0.24-0.66)	**<0.001**[b]	**<0.001**[b]	**0.002**[b]	**0.002**[b]
FIB-4‡	2.63	(1.68-4.42)	2.06	(1.52-2.75)	2.12	(1.44-2.64)	2.04	(1.39-2.69)	2.01	(1.28-3.29)	**0.001**[b]	**<0.001**[b]	**<0.001**[b]	**0.033**[b]

†Mean ± standard deviation; ‡ Median (P25-P75); p-value of the analysis using the stratum "before treatment" as a reference and comparing it with "week 4" * (p1), "end of treatment" ** (p2), "week 12 post-treatment" ** (p3) and "Week 24 post-treatment"(p4). [a]Test paired; the test of Wilcoxon signed posts. ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma-glutamyl transferase.

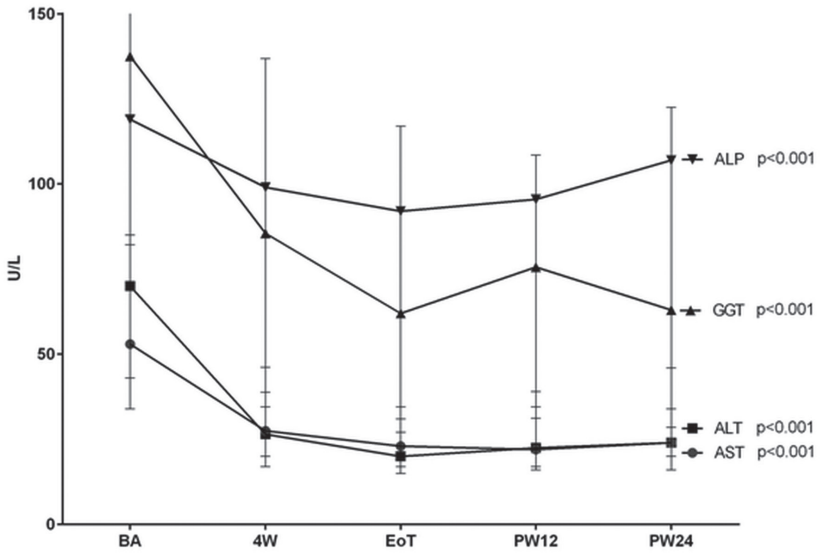

Figure 1 - Comparison of liver enzymes: ALP (UI/ L); GGT (UI/ L); ALT (UI/ L); AST (UI/ L), at different times: before treatment/ baseline (BA); week 4 of treatment (4W); end of treatment (EoT); week 12 post-treatment (PW12); and week 24 post-treatment (PW24) of 84 liver transplantrecipients with chronic hepatitis C, treated with sofosbuvir associated with daclatasvir or simeprevir. Comparisons made with Median and percentiles (P25-P75).

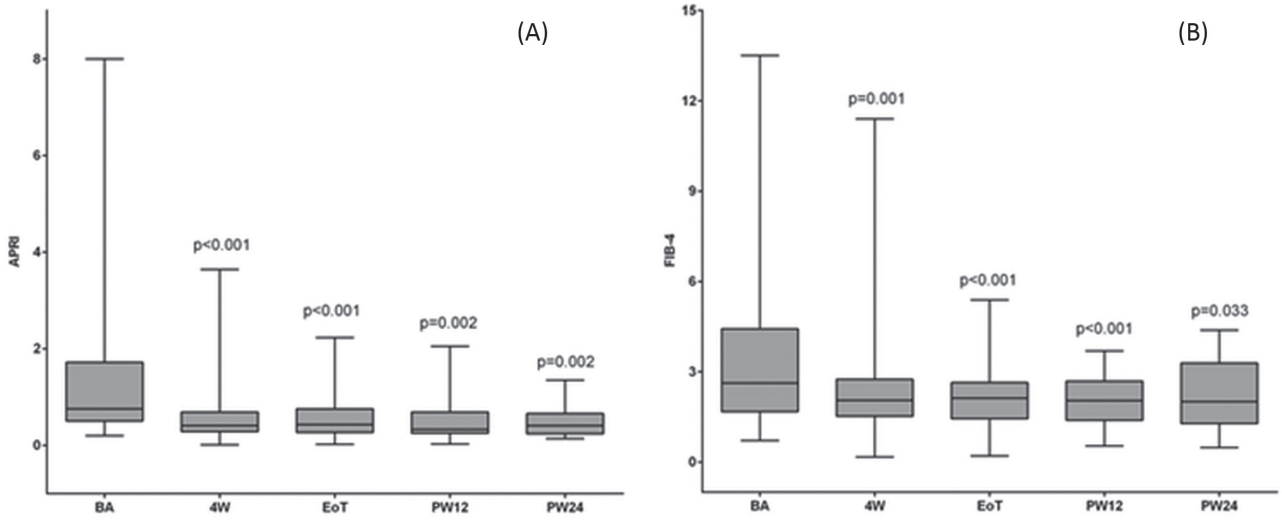

Figure 2 - APRI and FIB-4 values at different times: before treatment/ baseline (BA), week 4 of treatment (4W), end of treatment (EoT), week 12 post-treatment (PW12) and week 24 post-treatment (PW24) of 84 liver transplant recipients with chronic hepatitis C, treated with sofosbuvir associated with daclatasvir or simeprevir. (A) APRI. (B) FIB-4. Comparisons made with median and percentiles (P25-P75) with respect to baseline levels.

reported in a systematic review involving 885 transplanted patients, all of the cases associated with genotype 1, with higher SVR rates ($p < 0.01$) in patients with METAVIR F0-F2 (SVR = 97%, 95% CI: 0.93-0.99), when compared to patients with F3-F4 (SVR = 85%, 95% CI: 0.79-0.90)[32].

It is worth noting that some studies, including a meta-analysis, have shown a significant reduction in liver fibrosis after treatment of CHC with DAAs, both through the evaluation of fibrosis by elastography and APRI and FIB-4 scores in non-transplanted patients[34]. These data indicate a reduction in the progression of CHC to cirrhosis and, consequently, in the number of cases requiring liver transplantation as a consequence of HCV infection. However, there are few studies evaluating fibrosis regression after treatment of CHC with DAAs in liver-transplanted patients[35]. Indeed, Omar et al. found a progressive improvement in the degree of liver fibrosis, as indicated by elastography and serum scores, 12 and 18 months after the treatment of CHC with DAAs in 52 liver transplant patients[35]. The regression APRI and FIB-4 scores after treatment of CHC with DAAs, in both liver transplant and non-transplant patients, can occur by reducing serum levels of liver enzymes or by increasing the number of platelets[36].

Classically, patients with CHC have elevated serum levels of liver enzymes, reflecting virus-induced damage to hepatocytes, and normalization occurring during and after treatment, because of HCV elimination[36]. In fact, in our study, this expected behaviour of serum levels of liver enzymes was observed, starting at week 4 after the beginning of DAA therapy, reflecting a reduction in the necro-inflammatory activity in the liver parenchyma accompanying the elimination of HCV. We have also observed a progressive reduction in APRI and FIB-4 scores at 12 and 24 weeks after the end of the DAA therapy. The decrease in these scores must have occurred due to a reduction in serum aminotransferase levels, and the fact that there was no significant increase in the number of platelets, during or after antiviral therapy. The low increase in the number of platelets in our study was possibly due to the absence of thrombocytopenia in our patients before starting the therapy with DAAs, as was also seen in the study by Omar *et al.*[35]. Accordingly, in a study by Hsu *et al.*[36] in non-transplanted patients with CHC treated with DAAs, an increase in the number of platelets was observed only in patients who presented with thrombocytopenia before the treatment[36].

Side effects of DAAs, mostly mild, occurred in a quarter of our patients, with fatigue (8.3%) and headache (6.9%) being the most frequent. In only one patient, treatment was suspended due to an adverse effect (headache), coinciding with the finding of other authors claimed that DAAs have few side effects, requiring interruption of therapy in less than 2% of patients[19,37]. The main adverse reactions reported are headache, insomnia, fatigue, nausea and diarrhoea[38,39]. In a real life study by Bernuth *et al.*[40] including 37 liver transplanted patients with CHC treated with DAAs, few patients had anemia, which was related to the use of RBV. In only one patient, it was necessary to suspend the therapy due to the worsening of serum creatinine levels, which were already altered before the therapy with SOF + ledipasvir had started[40].

It is worth noting that the headache was the only cause of treatment interruption due to side effects in our study. The second case of failure resulted from a lack of adherence due to a lack of understanding of how to administer the therapeutic regimen. These two cases should be considered non-responders in the intention-to-treat assessment on the effectiveness of DAAs, although these patients did not complete the initial proposed therapy. These issues can occur in real life studies, and constitute limitations to treatment assistance, which can occur in medical practice.

Certainly, these failures in adherence to treatment could have been circumvented in a prospective and controlled study, highlighting the importance of clarifications and monitoring of patients before and during therapy with DAAs. Other limitations of our study that are worth considering are the small number of patients involved and the short follow-up time after the end of the antiviral treatment. In conclusion, treatment with second-generation DAAs achieved high SVR rates in a real life study, and was well tolerated by liver-transplanted patients with HCV in the Northeast of Brazil. Future studies with larger sample sizes will be necessary to confirm these findings.

ACKNOWLEDGMENTS

We would like to thank the Liver Transplant Unit of the Oswaldo Cruz Hospital of the University of Pernambuco (UPE) and the Hepatology Service/ General Transplant Unit (UGT) of the Professor Fernando Figueira Institute of Integral Medicine (IMIP), where the study was carried out, and the development agency Fundação de Amparo à Ciencia e Tecnologia of the State of Pernambuco (FACEPE) - Brazil.

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