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NEPHROLOGY - ORIGINAL PAPER

Efficacy and safety of the new antiviral agents for the treatment of hepatitis C virus infection in Egyptian renal transplant recipients

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Abstract

Purpose Hepatitis C virus (HCV) infection in kidney transplant recipients (KTRs) is common and can impact on patient and graft survival rates. The efficacy and safety of direct-acting antivirals (DAAs) to treat genotype-4 HCV-infected KTRs have not been fully established.

Methods A prospective, single-arm, single-center study was conducted at Mansoura Urology/Nephrology Center (Mansoura University, Egypt). 114 HCV RNA(+) genotype 4 KTRs were enrolled in this study after a hepatology consultation and consented to start treatment with interferon-free DAAs. A sofosbuvir-based regimen was given to 109 recipients that had creatinine clearance (Crcl) of > 30 mL/min/1.73 m^2. Ritonavir-boosted paritaprevir/ombitasvir was prescribed to five recipients with Crcl < 30 mL/min/1.73 m^2.

Results The mean age of the cohort was 45.2 ± 11.2 years; most were male. The mean duration with a transplant was 14.2 ± 3.5 years, with different immunosuppressive regimens, mostly based on calcineurin inhibitors. A rapid virological response (RVR), i.e., clearance of viral load, was achieved in 100% at 4 weeks after starting treatment. All patients had a sustained virological response (SVR) at 12 and 24 weeks posttreatment, with one exception. During DAA therapy serum creatinine increased in 12 patients. In three, this was concomitant with elevated calcineurin inhibitor and sirolimus trough levels. Graft biopsies were performed in 8 of these 12 patients: these revealed an acute rejection in 4 cases (acute cellular rejection grade-1A: $n = 2$, and grade-1B: $n = 2$). The rejection episodes occurred at 4–6 weeks after starting treatment.

Conclusion DAAs were highly efficacious and safely treated genotype-4 HCV-infected KTRs and had no significant adverse effects on graft function/survival.

Keywords Direct antiviral agents (DAA) · Drug interactions · HCV infection · Kidney transplantation · Sofosbuvir

Introduction

Chronic hepatitis C virus (HCV) infection can cause liver cirrhosis, liver failure, and hepatocellular carcinoma [1]. HCV infection is a major public health burden in Egypt, where it has the highest prevalence worldwide (18–20% of the population) [2].

Kidney transplant recipients (KTR) have better survival than those on long-term dialysis [3]. However, HCV infections are known to increase the risk of graft loss [4–6]. The prevalence of HCV among KTRs in developing countries ranges from 1.8 to 8% [7, 8]. Active HCV infections among KTRs are associated with shorter survival times due to cardiovascular complications, secondary infections, and accelerated liver fibrosis after long-term immunosuppressive therapy, when compared with non-HCV KTRs [9, 10].

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In the setting of kidney transplantation, in addition to the high risk of rejection associated with interferon alfa (IFN-a), a sustained virological response (SVR) is uncommon (13–43%) and there is high incidence of adverse events [11, 12]. Also, HCV treatments after kidney transplantation using ribavirin alone are unsuccessful [13–15].

First-generation DAAs, such as the protease inhibitors telaprevir and boceprevir, can be combined with alpha-interferon, but it is difficult to use this combination to treat HCV in KTRs. Thus, the recommendation of the Kidney Disease: Improving Global Outcome (KDIGO) group is to treat HCV-positive transplant candidates while they are still on dialysis [16].

With the recent advances in DAAs, new-generation DAAs (i.e., sofosbuvir combined with daclatasvir, simeprevir, or ledipasvir, with or without ribavirin) have been shown effective and safe at treating HCV in immune-competent patients [17–24]. Among KTRs, the use of a combination that includes at least two drugs from different DAA classes has resulted in an SVR at 12 weeks of > 90% [25–28]. However, a systematic evaluation on the efficacy and safety of DAAs to treat RTRs that have a genotype-4 HCV infection is still needed, especially among the Egyptian population, where 90% of cases are genotype-4.

Sofosbuvir is a prodrug: it is intracellularly metabolized and forms the active metabolite GS-461 203, followed by dephosphorylation resulting in the inactive compound GS-331 007, primarily renally excreted. Sofosbuvir is 85% bound to proteins. Daclatasvir, simeprevir, ledipasvir and 3D regimen extensively bind to plasma proteins (> 98%). Sofosbuvir and ledipasvir are not substrates or inhibitor/inducer of CYP3A4; conversely, simeprevir, daclatasvir, and 3D regimen are substrates of CYP3A4 [29]. Sofosbuvir, ledipasvir, simeprevir, and daclatasvir do not increase tacrolimus AUC. Conversely, the 3D regimen does, resulting in huge increase in tacrolimus AUC, thereby it has been recommended to give tacrolimus 0.5 mg every 7 days [29].

The aim of this study was to evaluate the efficacy and safety of DAA therapy in genotype-4 HCV(+) kidney transplant recipients in Egypt in a real-life setting.

Patients and methods

This was a prospective, single-center, non-controlled study.

Patients

Until December 2015, of the 2700 kidney transplant recipients that received a kidney transplant at the Urology and Nephrology Center in Mansoura University (Egypt), 394 (14%) had positive HCV antibodies. Of these, 114 (30%) were HCV RNA positive, i.e., above detection limit 15 IU/ml: these recipients were enrolled into this study and consented to receive DAAs.

Almost all the patients were recipients of live-related kidney transplants, i.e., 103 (90.3%). The others received live-unrelated kidney transplants. The mean donor age was 49.2 ± 12.3 years, 74% of them was females. None of the donors was hepatitis C positive before donation, as HCV-positive donors were excluded from donation before the era of DAAs. None of the recipients had donor-specific alloantibody pretransplant and all had a negative CDC crossmatch.

Post-transplant follow-up:

All kidney transplant recipients were followed up in the transplant clinic. They undergo close follow-up during the first year after transplantation, and thereafter every 3 months. During each visit, clinical evaluation is done and laboratory investigations in the form of serum creatinine, creatinine clearance, complete blood count, urine analysis and immunosuppressive trough levels are performed.

We do not monitor posttransplant for presence/absence of de novo DSA except in patients whose immunosuppression is steroid free.

Screening for BK virus is not routinely performed in our center except for patients with unexplained rise of serum creatinine: in this setting, we look for decoy cells and BKV PCR in blood and urine. If there is suspicion of BKV nephropathy, the kidney biopsy is then stained with anti-SV40.

Every CMV seronegative kidney recipient from a CMV-seropositive donor receives valganciclovir prophylaxis (900 mg/day adapted to eGFR) for the first 3 months post-transplant. CMV-seropositive kidney transplant patients receive instead valaciclovir 2 g bid (adapted to eGFR) for the first 3 months posttransplant. We do not routinely monitor for CMV DNAemia.

Immunosuppressive protocols

Sixty-six patients were maintained on tacrolimus-based immunosuppression, 30 patients received cyclosporine, and 20 patients received sirolimus-based immunosuppression. Of these, two patients were maintained on both tacrolimus and sirolimus; in addition, 70 patients received mycophenolate mofetil, and 20 patients received azathioprine. Fifty-five patients had received an induction therapy of basiliximab and 14 patients had received thymoglobulin as an induction therapy. The other 45 patients did not receive an induction therapy.

Direct-acting antiviral

Prior to treatment, a thorough evaluation was carried out, including laboratory investigations (serum creatinine, creatinine clearance, liver function tests (AST, ALT, gamma GT, total bilirubin), serum albumin, lipid profile, complete blood analyses, urine analysis, drug levels, antinuclear autoantibodies, and alpha-fetoprotein). Also, radiological assessments (pelvi-abdominal ultrasound and fibroscan) were performed on all patients. Echocardiography was ordered for some diabetic and cardiac patients.

After a hepatology consultation, all patients were treated for HCV with alpha-interferon-free DAAs. The combination of DAAs and treatment duration were determined according to prior HCV treatment history, hepatic decompensation, and creatinine clearance, as recommended by the EASL guideline, 2015 [27], and the protocol of the insurance institute in the Egyptian Ministry of Health. Patients with creatinine clearance (Crcl, MDRD formula) of > 30 mL/min/1.73 m^2 received a sofosbuvir-based regimen (109 recipients). The remainder, with Crcl < 30 mL/min/1.73 m^2, received a paritaprevir/ombitasvir/ritonavir (RTV/PTV/OMV) regimen ($n = 5$). Patients that had a previous relapse (11 patients) on or after alpha-interferon therapy received a triple therapy that included sofosbuvir, daclatasvir, and ribavirin.

The dose of ribavirin was calculated regarding creatinine clearance for each patient (patients with Crcl > 50 mL/min received 1200 mg/day, those with Crcl 30–49 mL/min received alternating dose, i.e., 200 and 400 mg every other day). The duration of treatment was 6 months for those that received a sofosbuvir-based regimen and 3 months for those on a paritaprevir/ombitasvir/ritonavir regimen. There was no change in daily dosage of any drug during therapy except for ribavirin, which was modified or stopped when hemoglobin levels dropped below 8.5 g/dL.

During the treatment period, all patients were monitored for liver function, renal function, hematological parameters, and immunosuppressive drug levels (tacrolimus, sirolimus, cyclosporine) every 2 weeks during the first month, and then monthly. Patients receiving RTV/PTV/OMV were monitored for kidney function and drug levels (tacrolimus, sirolimus, cyclosporine) twice weekly for 1 month, and then once weekly. Quantitative HCV PCR was performed for all patients prior to treatment, at 1 month after starting treatment, at the end of treatment, and at 12 and 24 weeks after completing treatment.

Finally, the daily doses of immunosuppressive drugs were not changed when starting DAA therapy when it was based on sofosbuvir therapy. Conversely, for paritaprevir/ombitasvir/ritonavir-treated patients, the tacrolimus dose was decreased to 0.5 mg/7 days, and cyclosporine dose was decreased to 20% of the maintenance dose; sirolimus was decreased to 1 mg/72 h. Indeed, these patients underwent frequent therapeutic drug monitoring.

Patients' data are available upon request.

Statistical analyses

All data were tabulated using an SPSS sheet. Descriptive measures were used for demographic and pre-treatment data. Repeated measure ANNOVA tests for parametric data and the Freidman test for non-parametric data were used to compare the laboratory findings before, during, and after treatment. A result was considered statistically significant if the p value was ≤ 0.05.

Results

A total of 114 HCV RNA (+) KTRs were included in this study. Their mean age was 45.2 ± 11.2 years and most were male (74.5%). Of the total, 12 recipients had received alpha-interferon treatment for HCV infection before transplantation. These patients had suffered from many complications when receiving alpha-interferon, especially myelosuppression and infections. In addition, none of these patients had achieved clearance of HCV after treatment.

Four patients had previously been co-infected with hepatitis B virus (HBV) and they received lamivudine therapy before transplantation; HBV PCR then became below detection limits. After transplantation, lamivudine was replaced by entecavir lifelong. None of them showed reactivation of hepatitis B during/after DAA therapy. One patient suffered from hepatocellular carcinoma and was under chemoembolization.

The baseline characteristics of the study population are summarized in Table 1. GFR was estimated using the MDRD equation [28]. The baseline radiological and clinical assessments of HCV-related hepatic condition are summarized in Table 2. Antinuclear autoantibodies were negative in all patients. Alpha-fetoprotein levels were within normal ranges for all patients.

Efficacy of treatment

A rapid virological response (RVR) was achieved in all patients after 4 weeks of receiving DAAs, regardless of if they were cirrhotic or not, and regardless of HCV genotype, the type of immunosuppressive protocol used, or the duration of transplantation. A sustained viral response (SVR) at 12 weeks and 24 weeks was successfully achieved in 113 patients (99.12%). The only relapse occurred at 3 months after completing treatment; the patient was on sofosbuvir and ribavirin. There was a significant improvement in liver

Table 1 Demographic data and associated medical disorders

Variable	Kidney transplant recipients (KTRs)
Age, years (mean ± SD)	45.2 ± 11.2
Gender: no. (%)	
Male	85 (74.5)
Female	29 (25.5)
Body mass index (mean ± SD), kg/m^2	23.8 ± 4.6
Previously treated by alpha-interferon, no. (%)	12 (10.5)
Comorbidities, no. (%)	
Hypertension	92/114 (83.6)
Diabetes mellitus	46/114 (40.3)
Ischemic heart disease	34/114 (29.8)
HBV co-infection	4/114 (3.5)
Cerebrovascular accident	4/114 (3.5)
Hepatocellular carcinoma	1/114 (0.8)
Kidney transplantation data	
Kidney transplant duration (mean ± SD)	14.2 ± 7.5 years
Baseline serum creatinine (mean ± SD)	1.3 ± 0.6 (mg/dL)
Baseline creatinine clearance (mean ± SD)	64.3 ± 31.3 (mL/min/1.73 m^2)
Biopsy results (before treatment):	
Previous acute rejection, no. (%)	60 (52.6)
Previous chronic rejection, no. (%	38 (33.3)

enzyme levels (ALT and AST) (p value = 0.000) with no significant hematologic effects (Table 3).

Safety

Treatment of HCV using DAAs was well tolerated with few adverse events. When hemoglobin levels became 8.5–9 g/dL, this was considered an indication for a dose reduction of ribavirin: this occurred in 11 patients. Of these, ten were receiving sofosbuvir + daclatasvir + ribavirin, and one was receiving ombitasvir + paritaprevir + ritonavir + ribavirin. Hemoglobin below 8.5 g/dL was considered an indication for cessation of ribavirin: this occurred in nine patients; of these five were receiving sofosbuvir + daclatasvir + ribavirin and four were receiving ombitasvir + paritaprevir + ritonavir + ribavirin. Two patients needed a blood transfusion.

Hemoglobin levels were corrected after ribavirin dose had been reduced by 70%. Hence, the mean hemoglobin for the 20 patients receiving ribavirin therapy was 8.06 ± 0.9 g/dL, whereas after ribavirin dose modification or its cessation, hemoglobin level increased significantly to 10.4 ± 0.86 g/dL (p = 0.0001, from an ANNOVA test). Consequently, this explains why there was no statistically significant difference regarding hemoglobin levels before, during, and after treatment (Table 3). Gastrointestinal upsets, dizziness, and bone aches were reported by six patients. Fatigue was a common complaint, reported by 17.5% of recipients (Table 4). The one recipient with known HCC before treatment was the only person that had a deteriorated liver function and decompensation at 3 months after completing treatment. Unfortunately, one case of malignancy was found: this was a non-Hodgkin lymphoma. He was diagnosed at 4 months after completing treatment and during routine investigations for vague abdominal pain.

Regarding graft function, there were no statistically significant differences regarding serum creatinine, creatinine clearance, tacrolimus, cyclosporine, and sirolimus trough levels before, during, or 4 weeks and 12 weeks after completing treatment (Table 5). However, serum creatinine was increased during DAA therapy in 12 patients; in 3, this was concomitant with elevated calcineurin inhibitor and sirolimus trough levels (the full data from these patients will be discussed later), especially associated with the RTV/PTV/OMV regimen. Graft biopsies were performed in 8 of these 12 patients: these revealed an acute rejection in 4 cases (acute cellular rejection grade-1A in 2 patients and grade-1B in 2 patients). The rejection episodes occurred at 4–6 weeks after starting treatment; the direct-acting antivirals were suspended during the rejection period (1–3 weeks) then resumed after serum creatinine was stable. The four patients maintained on tacrolimus had a mean trough level of 6.3 ± 0.6 ng/mL before a rejection episode and 4.4 ± 0.5 ng/mL at the time of rejection. The DAA regimen utilized for these four patients included sofosbuvir + daclatasvir + ribavirin. Graft biopsies revealed chronic transplant glomerulopathy in two patients during the last 2 weeks of treatment: the patients were maintained on cyclosporine. Acute tubular injury was diagnosed in two patients concomitant with an

Table 2 Clinical conditions associated with hepatic disease

Variable	114 kidney transplant recipients no. (%)
Fibroscan	
F0	49 (43)
F1	43 (37.7)
F2	13 (11.4)
F3	9 (7.9)
Liver ultrascan	
Normal	87 (76.3)
Enlarged	24 (21.05)
Cirrhotic	3 (2.65)
Spleen ultrascan	
Normal	98 (85.9)
Mild enlargement	12 (10.5)
Moderate enlargement	2 (1.8)
Marked enlargement	2 (1.8)
CHILD score	
CHILD A	99 (86.8)
CHILD B	11 (9.6)
CHILD C	4 (3.6)
HCV PCR	
Weak (< 100,000)	36 (31.5)
Moderate (100,000–800,000)	43 (37.7)
High (> 800,000)	35 (30.8)
HCV genotyping	
Genotype 1	15 (13.2)
Genotype 4	99 (86.8)
Clinical condition on starting treatment	
Lower limb edema	19 (16.7)
Ascites	4 (3.5)
Jaundice	3 (2.6)
Esophageal varices	4 (3.5)
History of hepatic encephalopathy	2 (1.8)
None	82 (71.9)

elevated trough level for tacrolimus in one patient and sirolimus in the other patient (Table 4).

To summarize the renal histological findings, two biopsies revealed tubulo-interstitial inflammatory infiltrates (acute cellular rejection 1A), two biopsies revealed tubulo-interstitial inflammatory infiltrates plus vasculitis (V1) (acute cellular rejection 1B), two biopsies revealed double contour of glomerular basement membranes in 25–50% of the section with peri-tubular capillary multi-layering, negative C4d staining and moderate degree of interstitial fibrosis and tubular atrophy (chronic transplant glomerulopathy); finally, the last two biopsies showed evidence of chronic calcineurin inhibitor nephrotoxicity. None of the eight biopsies had evidence for de novo focal segmental glomerulosclerosis or BKV nephropathy, i.e., SV40 staining was negative in all eight cases.

Calcineurin inhibitors and sirolimus trough levels were very high, with the RTV/PTV/OMV regimen (see below) requiring substantial dose reduction (Table 6). Otherwise, there was no significant difference with the other types of DAAs regarding CNI levels (Table 5). However, after completing DAA therapy the patients required overall higher daily immunosuppressants doses to achieve the same trough levels: this could reflect after HCV clearance improvement of hepatic function in metabolizing and excreting of these drugs.

Focus on patients treated with ritonavir-boosted paritaprevir/ombitasvir: pre-treatment data regarding the five patients that received RTV/OMV/PTV is illustrated in Table 6. One patient presented with an increase in ALT at 3 weeks after starting DAA therapy; baseline ALT was 34 IU/L, reaching a peak of 288 IU/L at 16 weeks after starting treatment. We did not stop RTV/OMV/PTV, and ALT thereafter started to improve and became normal by 6 weeks after completing DAA therapy (Fig. 1). Figure 2 highlights the correlation between graft function and immunosuppressive regimens utilized for patients received OMV + PTV + RTV regimen. Regarding graft function, there was a rise in serum creatinine in three patients (60%) (Fig. 2a). This rise was concomitant with high immunosuppressive trough levels. For tacrolimus (Fig. 2b), an acceptable trough level (4–8 ng/mL) was achieved at 10 days after a single starting dose of 0.5 mg in one patient, and treatment was continued successfully. For the other two patients, tacrolimus trough levels remained high for more than 21 days after a single dose equal to 0.5 mg of Prograf®, and the graft function was affected. One patient had perfect graft function before treatment, but then serum creatinine increased to 1.5 mg/dL from a baseline of 0.7 mg/dL. Thus, he was shifted to a sofosbuvir + daclatasvir regimen instead and was treated successfully. Regarding the other patient with impaired graft function at baseline, tacrolimus was suspended and he was maintained on a dual immunosuppressive regimen that included steroids and mycophenolate mofetil. Serum creatinine then dropped to basal levels (3.5 mg/dL) from a peak of 6.6 mg/dL, and the OMV + PTV + RTV regimen was continued successfully.

Cyclosporine trough levels had greater stability (Fig. 2c): the targeted level (75–150 ng/mL) was obtained at 48 h after starting a single dose. This was equal to 25% of the maintenance dose: DAA treatment was competed successfully.

For sirolimus (Fig. 2d), the targeted trough level (4–8 ng/mL) was obtained at 72 h after starting a single dose and was equal to 50% of the maintenance dose. However, within days following each single sirolimus there was a peak (> 30 ng/mL). After a few weeks, the patient was referred to us with sepsis after being over-immunosuppressed with high levels

Table 3 Laboratory investigations

	Before starting treatment	4 weeks after starting treatment	4 weeks after completing treatment	12 weeks after completing treatment (SVR 12)	24 weeks after completing treatment (SVR 24)	*p* value
Serum bilirubin, mg/dL (mean ± SD)	0.77 ± 0.3	0.68 ± 0.32	0.66 ± 0.29	0.66 ± 0.28	0.65 ± 0.24	0.47
Serum albumin, g/dL (mean ± SD)	3.77 ± 1.62	3.6 ± 0.6	3.66 ± 0.4	3.66 ± 0.37	4.4 ± 0.54	0.46
Serum cholesterol, g/dL (mean ± SD)	159.7 ± 52.9	165.1 ± 42.4	167.8 ± 38.21	166.6 ± 38.4	167.4 ± 38.4	0.85
ALT IU/L, med (min, max)	51 (11,145)	35 (10,160)	25 (7150)	20 (6155)	19 (6164)	**0.001**
AST IU/L, med (min, max)	53 (12,230)	35 (6.00,220)	28 (10,230)	24 (10,240)	24 (5230)	**0.001**
Hemoglobin, gm/dL (mean ± SD)	15.6 ± 2.2	11.7 ± 2.1	12 ± 2.1	12.2 ± 2.1	12.28 ± 2.2	0.23
White blood count, $*10^3/mm^3$ (mean ± SD)	7.4 ± 2.8	7.3 ± 2.7	7.6 ± 1.5	7.8 ± 2.4	7.9 ± 2.1	0.49
Platelet count, $*10^3/mm^2$ (mean ± SD)	191 ± 75	199 ± 73	206 ± 68	211 ± 62	216 ± 65	0.36

ALT alanine aminotransferase, *AST* aspartate aminotransferase, *med* median, *SD* standard deviation

Table 4 Treatments and complications

Variable	Kidney transplant recipients, no. (%)
DAA regimen	
Sofosbuvir + ribavirin	4 (3.55)
Sofosbuvir + daclatasvir	94 (82.45)
Sofosbuvir + ribavirin + daclatasvir	11 (9.6)
Ombitasvir + paritaprevir + ritonavir + ribavirin	5 (4.4)
Course duration	
Six months	109 (95.6)
Three months	5 (4.4)
Three- and six-month sustained viral response	113 (99.12)
Complication	
Anemia	20/114 (17.54)
Dizziness	6/114 (5.3)
Fatigue	20/114 (17.5)
Gastrointestinal upset	6/114 (5.3)
Bone aches	6/114 (5.3)
Hepatic decompensation	1/114 (0.8)
Hepatocellular carcinoma	0
Other malignancies (non-Hodgkin lymphoma)	1/114 (0.8)
Relapse	1/114 (0.8)
Graft-related complications	
Rise of serum creatinine ≥ 25% of baseline (%)	12/114 (10.5)
Need for a graft biopsy (%)	8/114 (7.0)
Acute rejection incidence (%)	4/114 (3.55)
Chronic rejection incidence (%)	2/114 (1.6)
Acute tubular injury incidence (%)	2/114 (1.6)

of sirolimus (the level reached > 30 ng/mL). After recovery from sepsis, the patient was shifted from OMV/PTV/RTV to sofosbuvir and daclatasvir.

Discussion

To the best of our knowledge, this is the first study conducted in the Middle East that has focused on the use of different DAAs given to kidney transplant recipients. In addition, this is to the best of our knowledge the largest study addressing DAA therapy in the setting of HCV (+) kidney transplant recipients. The selection of DAA and the length of treatment were based on EASL guidelines (2015) to treat HCV in kidney transplant recipients [27].

In our study, RVR was achieved in 100% of cases. HCV RNA became undetectable at 4 weeks after starting treatment. This was a better rate than that reported by Kamar et al. in a French population, where an RVR was achieved in 80% of patients [30]. There was no difference between cirrhotic and non-cirrhotic patients and achieving an RVR. This is in contrast to that observed by Lin et al., where non-cirrhotic patients achieved an undetectable viral load sooner than cirrhotic patients. However, not all of Lin et al.'s patients achieved an RVR within 4 weeks. This may have been due to race and/or genotype differences [31]. About 80% of their patients were genotype-1.

An SVR, which represents the standard measure for successful treatment as reported in the EASL guidelines (2015), is defined as undetectable HCV RNA at 12 weeks after completing treatment with DAAs [27].

SVRs at 12 and 24 weeks were successfully reached in 99.12% of our cohort. The SVR at week 12 in Kamar et al.'s

Table 5 Graft function and immunosuppressive drugs

	Baseline	4 weeks after treatment started	4 weeks after completing treatment	12 weeks after completing treatment	*p* value
Serum creatinine, (mg/dL) (mean ± SD)	1.32 ± 0.6	1.34 ± 0.66	1.31 ± 0.65	1.32 ± 0.23	0.232
Creatinine clearance (mL/min/1.73 m^2) (mean ± SD)	64.31 ± 31.33	62.33 ± 31.5	61.32 ± 30.42	62.42 ± 29.34	0.673
Tacrolimus dose (mg/day) (mean ± SD)	**6.4 ± 3.2**	**6.4 ± 3.2**	**7.9 ± 2.7**	**8.4 ± 2.6**	**0.0001**
Tacrolimus trough level (ng/dL) (mean ± SD)	4.4 ± 0.6	4.3 ± 0.5	4.1 ± 0.4	4.3 ± 0.6	0.325
Cyclosporine dose (mg/day) (mean ± SD)	**107.14 ± 44.98**	**92.86 ± 33.54**	**107.14 ± 44.98**	**114.28 ± 40.45**	**0.29**
Cyclosporine trough level (ng/dL) (mean ± SD)	90.1 ± 37.4	85.0 ± 31.38	81.6 ± 27.00	86.4 ± 25.2	0.328
Sirolimus dose (mg/day) (mean ± SD)	**4 ± 1.34**	**3.66 ± 1.56**	**4 ± 1.34**	**4 ± 1.34**	**0.4**
Sirolimus trough level (ng/dL) (mean ± SD)	8.35 ± 1.66	8.3 ± 1.57	8.2 ± 1.52	8.24 ± 1.83	0.354
Patients that needed tacrolimus dose reduction, no. (%)		3 (2.6%)			
Patients that needed cyclosporine dose reduction, no. (%)		1 (0.8%)			
Patients that needed sirolimus dose reduction, no. (%)		1 (0.8%)			

Table 6 Pre-treatment data regarding ritonavir-treated patients

Case #	1	2	3	4	5
Age (years)	38	24	20	56	34
Gender	Male	Female	Male	Female	Female
KTx duration (years)	18	10	2	17	5
Immunosuppressive regimen	St + Tac + MMF	St + Tac + MMF	St + Tac + MMF	St + CsA	Sir + MMF
SCr (basal) (mg/dL)	3.2	4.8	0.7	2.9	0.9
Crcl (mL/min)	22	13	101	18	98
Fibroscan	F1	F2	F1	F1	F1
CHILD score	A	B	A	A	A
Genotype	4	4	4	4	4
HCV PCR (IU/L)	8,569,450	856,664	784,586	458,635	2,587,695

KTx kidney transplantation, *SCr* serum creatinine, *Crcl* creatinine clearance, *HCV* hepatitis C virus, *St* steroids, *Tac* tacrolimus, *CsA* cyclosporine A, *MMF* mycophenolate mofetil, *Sir* sirolimus

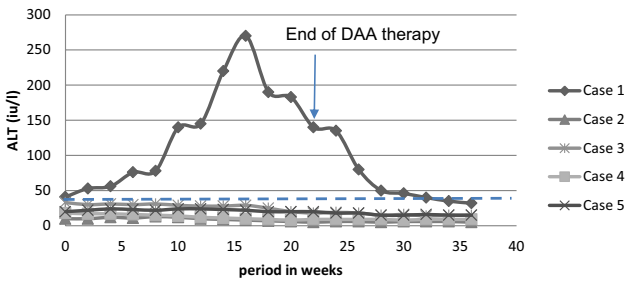

Fig. 1 Liver enzymes during treatment follow-up

study [30] was 100%, but the SVR at week 24 was only achieved in 8 of 25 patients (32%) as these were the only patients with available data at this point of follow-up. An American study by Sawinski et al. also reported a 100% SVR at week 12, but their study included a small number of patients (25 patients) and only a sofosbuvir-based regimen [32]. A short report by Goel et al. reported an RVR and SVR of 100%, but their sample size was only six KTRs [33].

In our study, liver enzyme levels, serum bilirubin, and serum albumin were assessed before, during, and after treatment. Only one patient had an increase in liver enzymes during treatment with RTV/PTV/OMV: overall, there was significant improvement. This result is similar to that reported in an Austrian report by Beinhardt et al. [34].

Anemia was only reported in recipients that also received ribavirin. Dose modifications were needed and erythropoietin was added until hemoglobin levels improved. Thus, there was no statistical significance regarding hemoglobin levels before, during, and after treatment. Reports by Bhamidimarri et al. [35] and Benjumea et al. [36] have advised not to use ribavirin. Apart from ribavirin, the other DAAs were well tolerated and caused no significant adverse effects, which is similar to the findings reported by Kamar et al., Sawinski et al., Bhamidimarri et al., and the PRISMA-compliant study

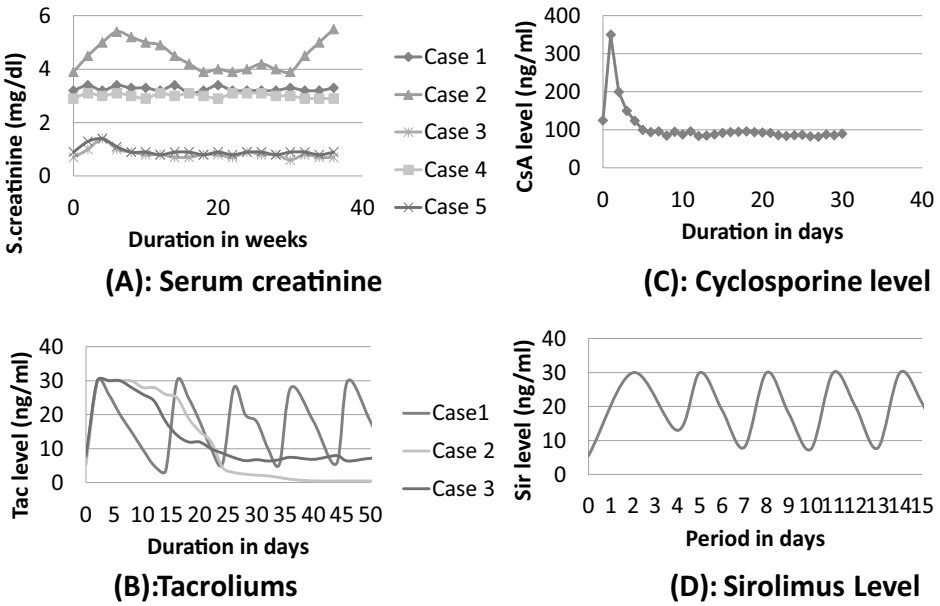

Fig. 2 Graft function and drug-level fluctuations during paritaprevir/ombitasvir/ritonavir treatment

by Chen et al. (Systematic Review on 6 studies including 360 KT patients) [30, 32, 35, 37].

Regarding graft function, there was a rise in serum creatinine in 12 patients (10.5%). Three cases were explained by high tacrolimus levels, which were attributed to a ritonavir-boosted regimen. At the end of treatment, serum creatinine had improved in 10 of 12 patients. Eight graft biopsies were performed: two biopsies revealed transplant glomerulopathy, four revealed an acute graft rejection (acute cellular rejection grade-1A in two patients and grade-1B in two patients), and two showed acute tubular injury. Bhamidimarri et al., reported increased serum creatinine in 4 of 25 patients with biopsy-proven antibody-mediated rejection and no improvement after completing treatment [35]. Goel et al. reported that two of six KTRs had graft impairment; biopsies revealed acute interstitial nephritis [33]. Kamar et al. observed a decline in GFR of ~ 10 mL/min/1.73 m^2 in 3 of 25 patients, but no biopsies were performed [30]. No graft rejection episodes were reported by Lin et al. [31]. Four of 20 KTRs showed increased serum creatinine in Sawinski et al.'s study, which resolved after reducing tacrolimus and stopping losartan [32].

Except for the five patients that received the RTV/PTV/OMV regimen, calcineurin inhibitors and sirolimus trough levels were not affected by drug interactions with the sofosbuvir regimens and there was no change in CNI or sirolimus dose during treatment. The same result was reported by Anand et al., who reported on liver transplant recipients maintained on tacrolimus with sofosbuvir and ribavirin [38]. Lin et al. also reported that CNI levels were stable throughout treatment [31]. Dose adjustments, mainly increases, were required for 13 of 25 patients in Bhamidimarri et al.'s study, especially after clearance of viral load and improvement in liver function tests [35]. Kamar et al. also reported a significant reduction in tacrolimus trough level during and after treatment, but no change in dose was required [30]. The dose of calcineurin inhibitors was changed for 45% of patients in Sawinski et al.'s study, with decreased tacrolimus levels [32]. This did not occur among our population, which may be attributed to recipients having worse liver conditions or a longer period of HCV infection.

In our study, aggressive dose reductions of CNI and sirolimus were needed for five patients that received a RTV/PTV/OMV regimen, Tacrolimus dose was reduced to 0.5 mg/week, cyclosporine dose was reduced to 20% of the maintenance dose every 48 h, and sirolimus dose was reduced to 50% of the maintenance dose every 72 h. The trough level was unstable throughout treatment and adverse events, such as infections and graft impairment, were reported. Belga and Doucette reported these changes in their article in 2016 [39]. These changes were also reported in eMedFusion, the Drug Interaction chart [40] in 2015, and by Badri et al. [41]. Benjumea et al. reported one case where ritonavir-boosted DAAs were given: however, treatment was discontinued because of worsening graft function caused by high tacrolimus levels [36].

Our study has some limitations. Firstly, it was observational and lacked the rigor of randomized controlled trials. We were also unable to assess the effect of DAAs on proteinuria at post-transplantation. In addition, the safety of sofosbuvir in cases that had low creatinine clearance was not tested. No protocol biopsies were performed during DAA treatment to detect any subclinical rejections. This study included a large number of Egyptian kidney transplant recipients that were maintained on various types of immunosuppressive drugs.

Conclusion

We conclude that the DAAs effectively treated HCV in these Egyptian kidney transplant recipients of which many were genotype-4 and some were cirrhotic: this outcome was regardless of the immunosuppressive regimen used and time since transplantation. Few adverse events affected the transplanted kidney and immunosuppressive drug trough levels were minimal.

Our recommendation is to treat kidney transplant recipients that have HCV with a sofosbuvir-based regimen. We think it better to avoid the use of DAA combinations that include ritonavir due to drug interactions with tacrolimus, cyclosporine, and sirolimus, which can lead to very high and unstable trough levels. This then requires aggressive dose reductions: patients then have a high risk of becoming over-immunosuppressed and developing drug toxicity to the graft.

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Compliance with ethical standard

Conflict of interest All authors declare no conflicts of interest.

Ethical standards This study was conducted according to compliance with ethical standards.

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