Immunological and biochemical parameters in patients with hepatitis C virus nephropathy before and after direct hepatitis C virus antiviral agents

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Background
Chronic hepatitis C is associated with multiple extrahepatic manifestations that may affect the infected patients. Egypt is the country with the highest incidence of hepatitis C virus (HCV) infection in the world. Hepatitis C-induced kidney disease is a devastating complication in patients with HCV infection. The development of multiple direct-acting antivirals (DAAs) has revolutionized the HCV infection treatment.

Aim
The aim was to determine the changes in immunological and biochemical parameters in patients with HCV nephropathy who receive DAA.

Patients and methods
The study included 30 patients diagnosed with HCV nephropathy according to the inclusion criteria and were tested for the immunological (C3, C4, rheumatoid factor) and biochemical parameters (urea, creatinine, urine analysis, 24 h proteins), before receiving their direct HCV antiviral agents for 12 weeks. Then participants were followed up regarding their immunological and biochemical parameters directly after termination of treatment.

Results
A total of 18 (60%) patients received sofosbuvir and daclatasvir with ribavirin, whereas 12 (40%) patients were on ombitasvir, ritnoprevir, and paritaprevir with ribavirin. Levels of transaminases were significantly improved after direct HCV antiviral agents in the current study. Significant improvement in the mean levels of blood urea and creatinine after direct HCV antiviral agents post-therapy when compared with their levels before treatment (49.16 ± 15.52 and 285.11 ± 39.07 mg/dl at pretreatment versus 25.44 ± 9.37 and185.03 ± 31.07 mg/dl after treatment respectively, with $P < 0.05$) with significant reduction in 24-h urinary protein following the antiviral therapy. Our study showed significant improvement in C3 and C4 levels after therapy with DAA. After 12 weeks of the therapy, all patients had negative HCV RNA by PCR at follow-up.

Conclusion
HCV infection is a major medical burden in patients with chronic kidney disease. However, HCV infection itself can cause chronic kidney disease. Effective antiviral agents play an important role in improvement of HCV-related nephropathy and other extrahepatic manifestations.

Keywords:
acute kidney injury, chronic hepatitis C, directly acting antiviral agents, estimated glomerular filtration rate, cryoglobulinemia, hepatitis-induced kidney disease, kidney disease, improving global outcomes

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Introduction

Hepatitis C virus (HCV) infection is a major public health problem, with an estimated three to four million people infected each year worldwide. More than 170 million people or 3% of the world population are chronically infected with HCV [1]. Hepatitis C (HCV) infection can induce kidney injury in four ways: (a) glomerular immune complex deposition; (b) direct viral invasion of the renal parenchyma; (c) renal complications of its extrarenal (e.g. hepatic) manifestations; and (d) nephrotoxicity of drugs used for its treatment [2]. The kidney is involved in 35–60% of patients with chronic hepatitis C. In 30% of cases, renal involvement begins with a nephritis syndrome and acute renal failure, whereas in 55%, there is only mild hematuria, microalbuminuria, proteinuria, and renal insufficiency [3]. The typical renal manifestations in HCV-infected patients include proteinuria, microscopic hematuria, hypertension, acute nephritic, and nephrotic syndrome. However, mostly the renal

disease is asymptomatic, and thus patients with HCV infection should be screened for proteinuria, hematuria, hypertension, and cryoglobulinemia [4]. All patients with HCV infection should be evaluated for the presence of albuminuria/proteinuria hematuria, decreased glomerular filtration rate (GFR), and hypertension. According to Kidney Disease Improving Global Outcomes guidelines, a random urine sample must be collected to determine the presence of hematuria and the albumin-to-creatinine ratio (ACR) or protein-to-creatinine ratio (PCR), together with the estimation of GFR (eGFR) annually in patients with HCV infection. The presence of an ACR greater than 30 mg/g or an eGFR less than 60 ml/min that persist for more than 3 months leads to the diagnosis of CKD [5]. The remarkable success of the current wave of direct-acting antiviral agents (DAAs) against HCV offers an unprecedented cure opportunity for millions of patients worldwide. Sustained viral response 12 weeks after completion of treatment (SVR12) is currently achievable in the vast majority, which predicts viral eradication in 98% of patients. This is associated with recovery from most clinical sequelae of HCV infection and provides a survival advantage to many subsets of patients, including reduction of cardiovascular mortality [6]. The DAAs suppress intracellular viral replication by inhibiting HCV proteins directly. DAAs are roughly classified into three categories: protease inhibitor, nonstructural proteins 5 A inhibitor, and polymerase inhibitor [7]. Egypt, the single country with the highest incidence of HCV infection in the world, has a difficult challenge, because (a) it is a unique country where the virus has its highest effect in the world [serological prevalence of 13.9–15.5% (14.7%), where positive nucleic acid test in 7.0–12.2% (9.8%) [3]]; (b) the prevalent viral strain in 90% of patients is genotype-4, which is typically difficult to treat, and on which only a few therapeutic trials have been published; and (c) the available health budget cannot accommodate the high demand for mass treatment [8,9].

Aim

The aim was to determine the changes in immunological and biochemical parameters in patients with HCV nephropathy who received DAAs.

Patients and methods

The current study is a cross-sectional observational study, conducted at Internal Medicine Department of Assiut University Hospitals in period between July 2016 and July 2017. To achieve our goal, 30 patients with HCV nephropathy with stage I, II, III, and IV CKD (eGFR <15 ml/min/m^2 calculated by MDRD equation) or patients with abnormal urinary finding, including proteinuria (>150 mg protein/24 h urinary collection or urinary ACR >3 mg/mmol in MSU sample), hematuria, or red cell cast in urine analysis, were included in the study after an informed consent. Patients with diabetes mellitus, HBV and HIV infections, lupus nephritis, polycystic kidney disease, congenital kidney diseases, advanced liver cirrhosis, hepatocellular carcinoma, obstructive uropathy, chronic pyelonephritis, and patients with end-stage renal disease (ESRD) on regular hemodialysis were excluded. Renal biopsy was done.

At the start of the study, patients were tested for serological blood tests (HCVab, HBsAg, and HIVAb by ELISA), PCR for HCV, complete blood count, liver function tests, blood urea nitrogen (BUN) and serum creatinine, coagulation profile (prothrombin time and concentration), urine analysis by dipstick and microscopic examination, 24 h urinary protein or ACR, C3 and C4 levels in serum by immunoturbidimetric method, rheumatoid factor by ELISA, glycated hemoglobin (HbA1C), and lipogram. Abdominal ultrasound and renal biopsy were also done for these patients.

Patients then received direct HCV antiviral agents for 12 weeks and they did not receive any immune suppressive, antiproteinuric drugs, or antibiotics during the duration of this study.

After 12 weeks, patients were tested for serological blood tests (HCVab, HBsAg, and HIVab by ELISA), PCR for HCV, liver function tests, BUN and serum creatinine, urine analysis by dipstick and microscopic examination, 24-h urinary protein or ACR, C3 and C4 levels in serum by immunoturbidimetric method, and rheumatoid factor by ELISA. All laboratory tests had been done at Assiut university hospitals by medical staff of clinical pathology department.

The study was approved by the local ethics committee in Faculty of Medicine, Assiut University.

Statistical analysis

Data were collected and analyzed using SPSS (statistical package for the social sciences, version 20; IBM, Armonk, New York, USA). Continuous data were expressed in the form of mean ± SD (range), whereas nominal data were expressed in the form of frequency (percentage).

χ^2-Test was used to compare the nominal data of different groups in the study whereas paired *t*-test

was used to compare mean of different variables before and after therapy. *P* value was significant if less than 0.05.

Results

The basics and laboratory data of our studied patients are shown in Table 1. Mean age ± SD was 52.18 ± 8.72 years, with range between 40 and 70 years. Of 30 patients included in the study, 21 (70%) patients were males and nine (30%) were females. Most patients (60%) came from rural areas. Most of them (70%) had no comorbidities, whereas four (13.3%), three (10%), and two (6.7%) patients had ischemic heart disease, chronic obstructive lung disease, and cerebrovascular stroke, respectively. A total of 18 (60%) patients received a regimen I of DAA (Sofosbuvir, Daclatasvir with ribavirin), whereas 12 (40%) patients were treated by regimen II (Ombitasvir, Ritnoprevir, and Paritaprevir with Ribavirin). All studied patients had positive HCV antibodies, positive HCV RNA by PCR, and positive rheumatoid factor, but they had negative HBsAg and HIV and negative antinuclear antibody with normal hemoglobin and normal abdominal ultrasound finding. All patients had negative HCV PCR 12 weeks after therapy.

In the current study, our patients had increased liver transaminases. Their mean levels were significantly improved after DAA therapy [mean alanine aminotransferase (ALT) level 72.23 ± 10.31 U/l before therapy vs. 21.73 ± 1.8 U/l after therapy, mean aspartate aminotransferase (AST) level 75.63 ± 13.92 U/l before treatment vs. 21.5 ± 1.4 U/l after therapy, with $P \leq 0.001$ for each]. However, post-DAA therapy level of bilirubin, serum albumin, total protein, and alkaline phosphatase had no significant differences from pretherapy level ($P > 0.05$), as shown in Table 2.

Table 3 showed that mean levels of serum creatinine were 3.23 ± 0.44 and 2.1 ± 0.35 mg/dl before therapy and after therapy, respectively, whereas that of blood urea nitrogen were 49.16 ± 15.52 and 25.44 ± 9.37 mg/dl, respectively, with significant improvement in their levels post-DAA therapy ($P > 0.05$). It was noticed that there was a significant reduction in 24-h urinary protein following the antiviral therapy (mean 24 h urinary protein at the start of therapy was 1597.34 ± 527.38 vs. 504.43 ± 214.14 mg at the end of therapy, with $P = 0.02$). Notably, all patients had albuminuria before starting the therapy; 13 (43.3%) and 17 (56.7%) patients had albuminuria ++ and +++, respectively, but with antiviral therapy, the degree of albuminuria was significantly decreased to 20 (66.7%) patients (18 (60%) and 2 (6.7%) patients with albuminuria + and ++, respectively, and 10 (33.3%) with no albuminuria, with $P = 0.03$), as shown in Table 3.

Table 4 showed significant improvement in the mean C3 and C4 levels after therapy with DAA (mean C3 and C4 were 0.92 ± 0.34 and 0.74 ± 0.45, respectively, before therapy versus 1.44 ± 0.49 and 1.23 ± 0.43, respectively, after DAA therapy with $P \leq 0.01$).

Table 1 Basics and laboratory data of the studied patients

Parameters	Patients (*n*=30) [*n* (%)]
Age (years)	
Mean±SD	52.18±8.72
Range	40–70
Sex	
Male	21 (70)
Female	9 (30)
DAA	
Regimen I	18 (60)
Regimen II	12 (40)
Laboratory investigations	
Positive HCV Ab	30 (100)
Positive HCV RNA by PCR	30 (100)
Negative HBsAg	30 (100)
Negative HIV	30 (100)
Positive rheumatoid factor	30 (100)
Negative ANA	30 (100)
Normal HGb A1C	30 (100)
Histopathological findings in renal biopsy	
Membrano-proliferative type I	15 (50)
Membranous GN	6 (20)
FSGS	5 (16.6)
Cryoglobulinemia	4 (13.4)

COLD, chronic obstructive lung diseases; CVS, cerebrovascular stroke data was expressed in form of frequency (percentage); DAA, directly acting antiviral agents; IHD, ischemic heart disease; regimen I, Sofosbuvir, Daclatasvir with ribavirin; regimen II, Ombitasvir, Ritnoprevir and Paritaprevir with Ribavirin, FSGS, focal segmental glomerulosclerosis, GN, glomerulo nephritis.

Table 2 The liver function tests pre-directly acting antiviral agents and post-directly acting antiviral agents therapy in the studied patients

Parameters	Pre-DAA therapy	Post-DAA therapy	*P*
Total bilirubin (mg/l)	0.81±0.16	0.84±0.15	0.91
Direct bilirubin (mg/l)	0.26±0.05	0.27±0.06	0.09
Alanine transaminase (U/l)	72.23±10.31	21.73±1.8	0.01 (S)
Aspartate transaminase (U/l)	75.63±13.92	21.5±1.4	0.00 (S)
Serum albumin (mg/dl)	34.96±1.4	35.5±1.5	0.11
Total protein (mg/dl)	85±4.8	82.3±5.6	0.32
Alkaline phosphatase (U/l)	123.98±9.8	113.08±5.67	0.21

Data were expressed in form of mean±SD and comparison was done by paired *t*-test.DAA, directly acting antiviral agent.*P* value was significant if <0.05.

Table 3 The kidney function tests pre-directly acting antiviral agents and post-directly acting antiviral agents therapy in studied patients

Parameters	Pre-DAA therapy	Post-DAA therapy	*P*
Blood urea nitrogen (mg/dl)	49.16±15.52	25.44±9.37	0.01
Serum creatinine (mg/dl)	3.23±0.44	2.1±0.35	0.001
eGFR	54.46±10.15	67.51±9.01	0.04
24 h urinary protein (mg)	1597.34±527.38	504.43±214.14	0.02
Albuminuria [*n* (%)]			0.03
Nil	0	10 (33.3)	
+	0	18 (60)	
++	13 (43.3)	2 (6.7)	
+++	17 (56.7)	0	

Data were expressed in form of mean±SD and comparison was done by paired *t*-test.DAA, directly acting antiviral agent.*P* value was significant if <0.05

Table 4 The mean of C3 and C4 levels in pre-directly acting antiviral agent and post-directly acting antiviral agent therapy level in studied patients

Parameters	Pretherapy	Post-therapy	*P*
C3	0.92±0.34	1.44±0.49	0.01
C4	0.74±0.45	1.23±0.43	0.01

Data were expressed in form of mean±SD and comparison was done by paired *t*-test.DAA, directly acting antiviral agent.*P* value was significant if <0.05.

Discussion

HCV infection is a systemic disorder which is often associated with a number of extrahepatic manifestations including glomerulopathies [10]. Large-scale community observational studies and others showed that HCV infection carries a risk for CKD and ESRD [11]. Several factors may contribute to the development of ESRD in HCV-infected patients. HCV may trigger a cascade of immune reactions that subsequently attack the kidneys and result in glomerulonephritis. HCV was also found to be associated with insulin resistance and dyslipidemia, thus, indirectly increasing the risk of renal disease [12]. The development of multiple DAA has revolutionized the HCV infection treatment, demonstrating cure rates higher than 90%, and showing less adverse effects than previous interferon-based regimens [13].

An important feature of HCV is that the virus avoids immune elimination, and this leads to chronic infection, accumulation of immune complexes, and autoimmune phenomenon at different extrahepatic tissues [14]. Improvement of HCV syndrome is closely related to SVR, which is subsequently related to decrease autoimmune response to HCV and reduced formation of immune complexes and cryoglobulins.

The present study was conducted in the period between July 2016 and July 2017 to determine the changes in immunological and biochemical parameters in patients with HCV nephropathy who received direct-acting antiviral agents. It was conducted at Internal Medicine Department of Assiut University Hospitals. Thirty patients with HCV nephropathy were enrolled in our study.

All patients were positive HCV Ab, positive HCV RNA by PCR, negative HBsAg, positive RF, and negative ANA and anti-ds DNA. DM was excluded by glycated hemoglobin, with no significant abnormalities presented in renal sonographic findings and confirmed diagnosis by renal biopsy. All patients had negative HCV RNA by PCR following 12 weeks after the therapy. Of 30 patients included in our study, 18 (60%) patients received sofosbuvir and daclatasvir with ribavirin and 12 (40%) patients used ombitasvir, ritnoprevir, and paritaprevir with ribavirin. Regarding demographic data of our studied patients, mean age (±SD) was 52.18 ± 8.72 years with range between 40 and 70 years, which was in agreement with study done by Golabi *et al.* [15]; thus, an age-based screening strategy is projected to identify a large number of geriatric (age >65) individuals with active viremia. Hospital admission rates are steadily increasing in the elderly HCV viremic population with higher inpatient charges, longer hospital stays, and more moderate/severe illness [15]. In our study, all patients achieved 12 weeks SVR, which was in agreement with the study of Ramos-Casals *et al.* [16], in which 50 patients were being treated with interferon (IFN)-containing regimens and 120 with IFN-free regimens. Slightly more patients treated with IFN-containing regimens had a complete clinical response (76 vs. 68%) and cryoglobulin clearance rates (56 vs. 47%), with a clearly lower rate of SVR (68 vs. 92%) compared with patients treated with IFN-free regimens [16]. In the case report of Obata *et al.* [17], IFN-free DAA therapy with DCV and ASV without immunosuppressive therapy resulted in a rapid virological response in case of a patient with HCV-associated cryoglobulinemic MPGN clinically manifested as RPGN, followed by an improvement in hematuria and proteinuria (complete remission of glomerulonephritis). In the study of Sise *et al.* [18], which analyzed treatment, efficacy, and safety of DAA, 8 of 12 patients (67%) received sofosbuvir paired with simeprevir, and four (33%) received sofosbuvir and ribavirin. Three received full weight-based dosing of ribavirin, and one required dose reduction owing to renal impairment. Most cases were treated with 12 weeks of therapy in comparison with our study. Of 30 patients included in our study, 18 (60%) patients received sofosbuvir and daclatasvir with ribavirin and 12 (40%) patients used ombitasvir,

ritnoprevir, and paritaprevir with ribavirin with 12 weeks of therapy. In agreement with our study, Sise *et al.* [18] showed ALT levels decreased from a median of 42 U/l (range: 18–207 U/l) pretreatment to 20 U/l (range: 10–41 U/l) on average after treatment (range: 12–24 weeks post-treatment), and albumin increased from 3.8 g/dl (range: 3.0–4.9 g/dl) to 4.0 g/dl (range: 3.7–4.9 g/dl) after treatment. In the study of Patel and colleagues significant reductions in ALT (65.3 vs. 24.6 IU/ml, $P < 0.001$) with DAA therapy was seen [18]. These results agree with our study that shows significantly improvement of ALT and AST after DAA therapy (mean ALT level 72.23 ± 10.31 U/l before therapy vs. 21.73 ± 1.8 U/l after therapy, mean AST level 75.63 ± 13.92 U/l before treatment vs. 21.5 ± 1.4 U/l after therapy with $P \leq 0.001$ for each, respectively).

Our study shows significant improvement in serum creatinine and blood urea nitrogen (serum creatinine 3.23 ± 0.44 and 2.1 ± 0.35 mg/dl pretherapy and post-therapy, respectively, whereas blood urea nitrogen was 49.16 ± 15.52 and 25.44 ± 9.37 mg/dl, respectively), with significant improvement in urea and creatinine after therapy ($P > 0.05$). These findings were also consistent with a significantly study of Bonacci *et al.* [19]. All patients in our study had albuminuria before starting the therapy [13 (43.3%) and 17 (56.7%) patients had albuminuria++ and +++, respectively] but with antiviral therapy albuminuria was significantly decreased to 20 (66.7%), 18 (60%), and two (6.7%) patients with albuminuria + and ++, respectively ($P = 0.03$). These findings were also consistent with a significant study by Bonacci *et al.* [19] which shows complete renal response in 71% (5/7) of participants with a significant improvement of eGFR (median = 40–54 ml/min/1.73 m^2, $P = 0.03$), decrease of proteinuria, and disappearance of hematuria ($P = 0.01$). Our study shows also significant improvement in C3 and C4 level after therapy with DAA. Mean C3 and C4 levels before therapy were 0.92 ± 0.34 and 0.74 ± 0.45, respectively, whereas their levels after DAA therapy were 1.44 ± 0.49 and 1.23 ± 0.43, respectively. This was in agreement with the study done by Bonacci *et al.* [19] which shows a complete normalization of the immune activation status. The variables associated with complete immunological response for both were IFN-based therapy, antiviral treatment duration, and RF. Patients treated for 24 weeks had a higher rate of cryocrit negativization (70% vs 44%; P ¼ .05), C4 improvement (75% vs. 48%; $P = 0.05$) and higher rate of complete immunological response [70% (14/20) vs. 37% (16/44); $P = 0.01$)] compared to those treated for 12 weeks. However, all immunological parameters improved in both groups 12 weeks after therapy. Among HCV-CV(CryoglobulinemicVasculitis) 42%, 71%, and 29% of patients presented normalization of C4, CH50, and RF levels, respectively. For the ACC (Asymptomatic Circulating Cryoglobulins patients) patients, the figures were 33%, 41%, and 33% [19,20].

Conclusion

HCV infection is a major medical burden in patients with chronic kidney disease. Moreover, HCV infection itself can cause chronic kidney disease. Effective antiviral agents play an important role in improvement of HCV-related nephropathy.

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Conflicts of interest

There are no conflicts of interest.

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