Impact of Hepatitis C Viral Load in Chronic Kidney Disease Patients

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Background and study aims: Chronic hepatitis C virus (HCV) infection is linked to chronic kidney disease (CKD) and hastens its progress to end-stage renal disease (ESRD). Previous studies have examined the association between chronic HCV and CKD and reported that HCV is correlated with proteinuria but not with low estimated glomerular filtration rate (eGFR) depending mainly on anti-HCV antibodies to diagnose chronic HCV infection. However, it is the HCV viral load to diagnose active HCV infection. Therefore, our study aimed to elucidate the relationship between HCV viral load and CKD.

Patients/Material and Method: It's a cross-sectional observational study that included 204 subjects that were classified into group 1 (90 chronic HCV patients without a history of chronic kidney disease) and group 2 (114 chronic HCV patients with a history of chronic kidney disease). All subjects underwent full history taking, medical examination, and laboratory investigation from May 2017 to May 2019.

Results: In CKD patients, HCV viral load was correlated with age, platelet count, serum creatinine, eGFR, and serum bilirubin. Higher HCV viral load was one of the significant predictors of low eGFR in patients with chronic HCV infection in addition to the female sex, older age, lower hemoglobin, higher international normalized ratio, and higher alanine transaminase.

Conclusion: There is a strong positive association between HCV viral load and serum creatinine in CKD patients. Higher HCV viral load carries a greater risk for lower eGFR in patients with chronic HCV infection .

INTRODUCTION

Over 170 million people are chronic infected by hepatitis C virus (HCV) and about 3 to 4 million individuals are newly infected every year **[1]**. Hepatic manifestations are the most common among HCV patients. However, 40% to 74% of patients have extrahepatic manifestations, especially glomerulonephropathy **[2]**. Chronic HCV infection is linked to chronic kidney disease (CKD) and hastens its progress to end-stage renal disease (ESRD) **[3]**. Furthermore, HCV infection augments complications in dialysis patients and kidney transplant recipients [4]. Several researchers have studied the association between chronic HCV and CKD and disclosed that HCV is correlated with proteinuria but not with a low estimated glomerular filtration rate (eGFR) **[5]**. However, previous researchers mainly used anti-HCV antibodies to diagnose chronic HCV infection. Nevertheless, it is the HCV viral load to diagnose active HCV infection **[6]**. Our study aimed to elucidate the relationship between HCV viral load and CKD and determine predictors of low eGFR in chronic HCV patients.

PATIENTS/MATERIAL AND METHODS

This was a cross-sectional observational study from May 2017 to May 2019.

We included patients with a history of chronic HCV infection after taking their informed written consent to participate in the study. Exclusion criteria were uncontrolled hypertension, active infection, ischemic heart disease, cerebrovascular stroke, inflammatory disorders, malignancy, thyroid disorders, and pregnant patients.

The subjects were categorized into:

- **Group 1:** It included 90 chronic HCV patients without a history of chronic kidney disease. They were 39 males and 51 females, and their ages ranged from 19 to 76 years with a mean ± SD of 40.2 ± 16.04 years.
- **Group 2:** It included 114 chronic HCV patients with a history of chronic kidney disease. They were 65 males and 49 females, and their ages ranged from 20 to 82 years with a mean ± SD of 55.68 ± 13.75 years.

Throughout the follow-up period from May 2017 to May 2019, all research participants were subjected to the following:

- A comprehensive history was obtained with stress on age, sex, drug history, and the prevalence of other medical disorders, especially diabetes, hypertension, and chronic kidney disease.
- Clinical examination included vital signs, weight, height, and features of jaundice, pallor, or bleeding tendency.
- Laboratory investigations

a) Routine investigation: Blood samples were collected after overnight fasting for 8-12 hours. After skin sterilization with the ethyl alcohol swap, 10 ml of peripheral venous blood was withdrawn from each patient. Investigations included complete blood count (CBC), serum total bilirubin, aspartate transaminase (AST), alanine transaminase (ALT), serum albumin, total plasma protein, alpha-fetoprotein, blood urea, serum creatinine, fasting blood sugar (FBS), serum uric acid, prothrombin time, partial thromboplastin time, international normalized ratio (INR), and estimated glomerular filtration rate (eGFR), which determined using the modification of diet in renal disease (MDRD) formula: $186 \times (\text{Creatinine}/88.4)^{-1.154} \times (\text{Age})^{-0.203} \times (0.742 \text{ if female}) \times (1.210 \text{ if black})$.

b) Special investigation: HCV RNA PCR viral load test. Quantitative HCV RNA was measured using Amplicor 2.0 (Roche Diagnostics, Meylan, France).

Data processing was carried out using version 26 of the SPSS (Statistical Package for Social Sciences) program. We described quantitative variables using means and standard deviations and categorical variables by absolute frequencies. To check hypotheses for use in parametric experiments, the Kolmogorov-Smirnov and Levene tests were used. The independent sample Student's t-test was used to compare two groups of normally distributed data while Mann Whitney U test was used for non-normally distributed data. To calculate the association between two continuous variables, the Spearman correlation coefficient was used. The level of statistical significance has been determined at 5% ($P < 0.05$). A highly significant difference was present if $p \leq 0.001$. Independent predictors for eGFR were determined using logistic regression analysis. All variables found significant on univariate logistic regression with a $p < 0.05$ were studied for multiple regression analysis.

RESULTS

The study included 204 subjects that were classified into three groups. They were matched regarding sex as shown in table 1. Other demographic characteristics varied significantly among the studied groups and group 2 had significantly older age, higher risk of hypertension (HTN) and diabetes mellitus (DM) than group 1 as presented in table 1. In addition, we encountered significant differences between the studied groups regarding laboratory data and group 2 had significantly lower hemoglobin, higher INR, higher ALT, lower serum albumin, higher serum creatinine, lower eGFR and higher HCV viral load than group 1 as presented in table 2. Additionally, we found that in group 1 high HCV viral load (> 800 000 IU/mL) was strongly positively correlated with the female sex, weight, BMI, while it was negatively correlated with serum albumin, and serum bilirubin. In group 2, we found that high HCV viral load (> 800 000 IU/mL) load was positively correlated with age, platelet count, serum creatinine, while it was negatively correlated with eGFR, and serum bilirubin as shown in table 3.

Female sex, older age, hemoglobin, INR, HCV viral load (> 800 000 IU/mL), ALT, and serum

albumin represented the significant predictors of low eGFR in chronic HCV patients on univariate analysis of all risk factors in our patients' groups as shown in table 4. Finally, after fixing of all non-significant risk factors on multivariate analysis, we found that HCV viral load (> 800 000 IU/mL) is one of the significant predictors of low eGFR in chronic HCV patients (P= 0.003) as shown in table 5.

Table (1): Comparison of the studied groups regarding demographic data.

Variable	Group 1	Group 2	p-value
Age (years)	40.2±16	55.68±13.7	<0.001**
Sex Male Female	 39 (43.3) 51 (56.7)	 65 (57) 49 (43)	 0.053
Weight (kg)	73±12.55	76.9±15.7	0.073
BMI (kg/m^2)	27.3±4.9	28.5±5.98	0.148
HTN	0 (0)	13 (11.4)	<0.001**
DM	0 (0)	25 (21.9)	<0.001**

Data are presented as number (%) or mean ± standard deviation, (**): Highly significant, (BMI): Body mass index, (HTN): Hypertension, (DM): Diabetes mellitus.

Table (2): Comparison of the studied groups regarding laboratory data.

Variable	Group 1	Group 2	p-value
Hemoglobin (g/dL): *Mean ± SD*	13.09±1.84	11.88±1.65	<0.001**
WBC (x10^3/mm^3): *Mean ± SD*	7.4±1.8	7.34±1.97	0.728
Platelets (x10^3/mm^3): Median (Range)	213.5 (102-344)	205.5 (79-819)	0.055
INR: *Mean ± SD*	1.03±0.063	1.1±0.14	<0.001**
Bilirubin (mg/dL): *Mean ± SD*	0.7±0.34	0.73±0.29	0.126
ALT (U/L): Median (Range)	27 (16-68)	31 (10-234)	0.012*
Albumin (g/dL): *Mean ± SD*	4.3±0.38	4.09±0.47	<0.001**
Creatinine (mg/dL): Median (Range)	0.6 (0.4-0.8)	2.96 (0.7-13)	<0.001**
eGFR (ml/min/1.73 m^2): Median (Range)	135.5 (101.2-272.2)	19.55 (4.3-91.2)	<0.001**
HCV PCR (IU/mL): Median (Range)	179 374 (2 200-3 020 977)	520 000 (3 320-9 571 000)	<0.001**

(Sig): Significance, (**): Highly significant, (WBC): White blood cells, (INR): International normalized ratio, (ALT): Alanine transaminase, (eGFR): Estimated glomerular filtration rate.

Table (3): Correlation between HCV viral load and selected study parameters in groups 1 and 2.

Variables	Group 1		Group 2	
	r	p-value	r	p-value
Female sex	+0.284	0.007**	-0.131	0.166
HTN	-	-	+0.089	0.347
DM	-	-	-0.015	0.870
Age	+0.108	0.311	+0.251	0.007**
Weight	+0.281	0.007**	+0.156	0.097
BMI	+0.417	<0.001**	+0.105	0.267
Hemoglobin	+0.075	0.485	+0.092	0.328
WBC	-0.016	0.877	+0.107	0.256
Platelets	-0.006	0.954	+0.188	0.045*
INR	-0.122	0.253	+0.003	0.978
eGFR	-0.110	0.301	-0.222	0.018*
Bilirubin	-0.214	0.042*	-0.271	0.004**
Creatinine	+0.107	0.314	+0.231	0.013*
ALT	+0.139	0.191	-0.034	0.718
Albumin	-0.224	0.034*	+0.068	0.469

(r): Spearman's rank correlation coefficient, (*): Significant, (**): Highly significant, (HTN): Hypertension, (DM): Diabetes mellitus, (BMI): Body mass index, (WBC): White blood cells, (INR): International normalized ratio, (ALT): Alanine transaminase, and (eGFR): Estimated glomerular filtration rate.

Table (4): Univariate logistic regression of low eGFR (<60 ml/min/1.73 m^2) in groups 1 and 2.

Variables	β	SE	OR	95% CI	p-value
Female sex	+0.716	0.285	2.045	(1.171-3.574)	0.012*
Age	+0.055	0.010	1.057	(1.036-1.078)	<0.001**
Weight	+0.012	0.010	1.012	(0.992-1.031)	0.235
BMI	+0.009	0.025	1.009	(0.960-1.060)	0.716
Hemoglobin	-0.411	0.090	0.663	(0.556-0.791)	<0.001**
WBC	+0.000	0.074	1.000	(0.865-1.156)	0.997
Platelets	-0.001	0.002	0.999	(0.995-1.002)	0.512
INR	+4.293	1.428	73.161	(4.457-1200)	0.003**
HCV PCR	+0.544	0.167	1.723	(1.242-2.390)	0.001**
Bilirubin	+0.197	0.453	1.217	(0.501-2.960)	0.664
ALT	+0.027	0.009	1.027	(1.009-1.047)	0.004**
Albumin	-0.797	0.331	0.450	(0.236-0.861)	0.016*

(β): regression Coefficient, (SE): standard error, (OR): Odds Ratio, (95%CI): 95% confidence interval, (*): Significant, (**): Highly significant, (BMI): Body mass index, (WBC): White blood cells, (INR): International normalized ratio, (ALT): Alanine transaminase, and (eGFR): Estimated glomerular filtration rate.

Table (5): Multivariate logistic regression of low eGFR (<60 ml/min/1.73 m^2) in groups 1 and 2.

Variables	β	SE	OR	95% CI	p-value
Female sex	+0.749	0.372	2.115	(1.021-4.381)	0.044*
Age	+0.048	0.012	1.049	(1.025-1.074)	<0.001**
Hemoglobin	-0.529	0.116	0.589	(0.470-0.739)	<0.001**
INR	+4.640	1.713	103.514	(3.604-2973)	0.007**
HCV PCR	+0.624	0.209	1.866	(1.238-2.813)	0.003**
ALT	+0.028	0.011	1.028	(1.006-1.051)	0.014*
Albumin	+0.136	0.404	1.145	(0.519-2.528)	0.737

(β): regression Coefficient, (SE): standard error, (OR): Odds Ratio, (95%CI): 95% confidence (*): Significant, (**): Highly significant, (INR): International normalized ratio, and (ALT): Alanine transaminase.

DISCUSSION

A known cause for the progression of CKD to ESRD is HCV infection, which is followed by shorter survival in CKD patients **[2]**. Additionally, the frequency of HCV infection is higher among CKD patients than in the general population, and it is principally higher in hemodialysis patients **[7]**. Furthermore, HCV infection augments complications in dialysis patients and kidney transplant recipients **[4]**.

Our study included 204 subjects that were classified into group 1 (90 chronic HCV patients without a history of chronic kidney disease) and group 2 (114 chronic HCV patients with a history of chronic kidney disease).

Regarding the comparison of the demographic data of the studied groups, group 2 had a significantly older age than group 1 because the elderly are exposed to cardiovascular risk factors, high blood pressure, and diabetes for life, and these diseases may also damage the kidneys **[8]**. Group 2 also had a higher risk of hypertension (HTN) and diabetes mellitus (DM) than group 1, since these conditions are well-known risk factors for kidney disease initiation and progression **[9]**.

Regarding the comparison of the laboratory data of the studied groups, group 2 had significantly lower hemoglobin than group 1. That result against that obtained by Tsai et al **[10]**. It can be due to relative erythropoietin insufficiency, elevated hepcidin levels, reduced erythrocyte lifespan, and disturbed iron metabolism **[11]**. INR was significantly higher in group 2 than in group 1 because CKD affects clotting function and causes bleeding tendency **[12]**. In addition, group 1 and had higher ALT and lower albumin than group 2 due to chronic necroinflammatory damage of liver cells by HCV **[13]**. These results agree with Afify et al. **[13]** and Fujita et al. **[14]**. Finally, group 2 had a significantly higher HCV viral load than group 1, which may be because

the higher HCV viral load can cause more significant glomerulopathy. Additionally, CKD patients are immunocompromised, which raises the risk of infection [15].

Our study showed that HCV viral load in group 1 was strongly correlated with female sex, weight, BMI, serum albumin, and serum bilirubin. In group 2, HCV viral load was strongly correlated with age, platelet count, eGFR, serum creatinine, and serum bilirubin. In CKD patients, the strong association between HCV viral load and old age can be explained by that elderly have higher exposure to oxidative stress, lower hepatic blood flow, diminished mitochondrial function, and weakened immunity **[16]**. Additionally, the platelet count was strongly positively associated with HCV viral load in CKD patients, as a higher platelet count may suggest malnutrition inflammation cachexia syndrome (MICS) with an abnormal immune response in CKD **[17]**. Moreover, the strong negative correlation between HCV viral load and serum bilirubin in CKD patients may be explained by that the lower serum bilirubin loses its protective anti-inflammatory effects in CKD patients, causing further renal damage and resulting in higher HCV viral load **[18]**. Additionally, HCV viral load was strongly correlated with eGFR and serum creatinine in CKD patients and these results agreed with Kim et al **[19]**. Finally, we noticed that the HCV viral load in CKD patients did not affect the liver condition, but negatively affected the renal condition.

The negative association between HCV viral load and serum creatinine may be either a sequence of immunocompromised state in CKD or a causal relationship as HCV infection can result in glomerular damage by causing extrahepatic manifestations including membranoproliferative glomerulonephritis, cryoglobulinemia, focal segmental glomerulo-sclerosis, renal thrombotic microangiopathy, and membranous nephropathy **[20]**.

Our results showed that the probability of low eGFR (<60 ml/min/1.73 m^2) in groups 1 and 2 by univariate binary logistic regression was significantly greater with female sex [odds ratio (OR): 2.045, 95% confidence interval (CI): 1.071- 3.574 and P = 0.012], older age [OR: 1.057, 95% CI: 1.036-1.078 and P = <0.001], lower hemoglobin [OR: 0.090, 95% CI: 0.556-0.791 and P = <0.001], higher INR [OR: 73.1, 95% CI: 4.457-1200 and P = 0.003] higher HCV viral load (> 800 000 IU/mL) [OR: 1.723, 95% CI: 1.242-2.390, P = 0.001], higher serum ALT [OR: 1.027, 95% CI : 1.009-1.047, P = 0.004], and lower serum albumin [OR: 0.450, 95% CI: 0.236-0.861, P = 0.016]. Analysis of the significant predictors of low eGFR (<60 ml/min/1.73 m^2) in group 1 and group 2 in our study as identified by univariate binary logistic regression was done using multivariate logistic regression after fixing all other non-significant variables. The analysis showed that the probability of low eGFR was significantly higher with female sex, older age, hemoglobin, INR, HCV viral load (> 800 000 IU/mL), and ALT.

The drawbacks of our research are that the study involved a comparatively limited number of patients with CKD, the status of liver fibrosis was not determined and the real time of acquisition of HCV infection was not assessed.

CONCLUSION

This cross-sectional study showed that HCV viral load was negatively associated with eGFR in chronic HCV patients. The relationship was significant, when chronically HCV-infected patients had chronic kidney disease. Higher HCV viral load (> 800 000 IU/mL) represents one of the important predictors of low eGFR (<60 ml/min/1.73 m2) in chronic HCV patients.

List of Abbreviations:

Hepatitis C Virus (HCV), Chronic kidney disease (CKD), End-stage renal disease (ESRD), Estimated glomerular filtration rate (eGFR), Complete blood count (CBC), Aspartate transaminase (AST), Alanine transaminase (ALT), Fasting blood sugar (FBS), International normalized ratio (INR), Modification of diet in renal disease (MDRD), Statistical Package for the Social Sciences (SPSS), Hypertension (HTN), Diabetes mellitus (DM).

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