Effects of concomitant hepatitis C virus infection in patients with underlying lupus nephritis on long-term renal outcome

Ahmed H. Mitwalli, Ashik Hayat, Jamal Alwakeel and Durdana Hammad

Department of Medicine, Division of Nephrology, King Saud University, King Khalid University Hospital, Riyadh, Saudi Arabia

Correspondence and offprint requests to: Ashik Hayat; E-mail: ashikhayat@hotmail.com

Abstract

Background. Despite recent advances in the management of lupus nephritis (LN), these unfortunate patients are at a higher risk of developing chronic kidney disease (CKD). Concomitant chronic hepatitis C virus (HCV) infection is associated with adverse outcome in patients with LN and further compounds the risk as some of these patients choose to undergo kidney transplantation in the near future. Objectives. The aim of the present study is to evaluate the long-term impact of chronic HCV infection in patients with underlying Class IV LN on renal function, progression to end-stage renal disease (ESRD) and patient survival. Methods. Retrospective analysis of the medical records of 134 nondialysis-dependent patients with biopsy-proven World Health Organization Class IV LN with chronic HCV infection was done from January 1995 to January 2008 at King Khalid University Hospital, Riyadh, Saudi Arabia. Primary and the secondary end points were death or the development of ESRD. The patients were followed over a period of 6.7 ± 3.3 (1–14.4) years. Results. From a total of 134 biopsy-proven Class IV LN patients, 15 (11.2%) patients were HCV positive of which 2 (13.3%) patients were male and 13 (86.7%) patients were female. One hundred and nineteen (88.8%) patients were HCV negative of which 17 (14.3%) were male and 102 (85.7%) were female. The mean age was 32.47 ± 11.8 years. Eight (53.3%) patients in the HCV-positive group versus 19 (22.6%) patients in the HCV-negative group progressed to severe renal impairment with serum creatinine > 350 µmol/L (P = 0.024). A total of 8 (53.3%) patients in the HCV-positive group versus 18 (17.3%) in HCV-negative group progressed to ESRD (P = 0.005). The mean creatinine clearance was higher (43.3 ± 33 mL/min) in the HCV-negative LN group at last follow-up than in the HCV-positive patients (25 ± 34.9 mL/min) with a statistically significant P-value of 0.0463. Five patients (33.3%) with HCV-positive LN died in comparison to eight (7.6%) patients who were HCV negative P = 0.03; however, the cause of hospital mortality was mainly cardiovascular disease (CVD) and infection and none of the patients died of chronic liver disease, although there was significant deterioration of the liver function at the end of the study. Kaplan–Meier survival estimates showed a significantly inferior renal function and rapid deterioration to ESRD in LN patients with concomitant HCV infection, with a dialysis free survival of 95 and 80% for the HCV-negative group and 90 and 68% for the HCV-positive groups at the end of 5 and 10 years respectively, with a highly significant P-value of <0.05 at the end of 10 years. Conclusion. The present study highlights that concomitant HCV infection in patients with LN is associated with worse renal outcome, higher rate of progression to ESRD and reduced patient survival.

Keywords: end-stage renal disease; hepatitis C virus infection; lupus nephritis; renal outcome

Introduction

Chronic hepatitis C virus (HCV) infection is a global health problem affecting >170 million people all over the world and is responsible for >1 million deaths due to cirrhosis and hepatocellular carcinoma [1, 2]. Multiple extra hepatic manifestations including kidney disease can be caused by HCV infection [3]. Both chronic HCV infection and chronic kidney disease (CKD) are common and are potentially serious medical health problems throughout the world and in recent years, it has become clear that these two conditions are linked in several important ways [4–7]. HCV infection is said to be a silent killer and is associated with increased cardiovascular mortality in end-stage renal disease (ESRD) patients on hemodialysis (HD) [8–11]. In a large study of 448 renal transplant patients, Mitwalli et al. [12] found that HCV infection was a major predictor of adverse renal outcome and negatively influenced graft and patient survival.

Despite improvements in the management of lupus nephritis (LN), these patients remain at a high risk of developing ESRD with an estimated incidence of 15% [13–16]. Since some immunosuppressive medications used for treating the LN patients inhibits cell-mediated immunity, these patients are prone to viral infection and in turn viral proliferation and liver damage. The Kidney Disease: Improving Global Outcomes guidelines recommend that all patients with CKD and those initiating HD should be routinely screened for HCV infection [17]. Although clinical guidelines regarding the management of chronic HCV...
infections in patients with systemic lupus erythematosus have been published in 2009, there is still a lack of data regarding the long-term impact of HCV infection in patients with underlying LN [18, 19].

Materials and methods

Study design and study population

This retrospective observational study was carried at the King Khalid University hospital affiliated with the King Saud University, Riyadh, between January 1995 and January 2008.

Inclusion criteria

New incident biopsy proven Class IV LN patients aged >18 years were included into the study, all patients who had previously received dialysis or were currently on dialysis were excluded from the study. Complete history including age, sex, ethnicity and comorbidities e.g. hypertension, diabetes mellitus, chronic obstructive pulmonary disease, stroke or history of coronary artery disease were recorded at the time of enrollment into the study. A complete physical examination including pulse, blood pressure (BP), postural drop, peripheral edema, body mass index and systemic examination were also recorded at the time of enrollment into the study. The laboratory parameters including complete hemogram, renal function tests like blood urea, serum creatinine, serum calcium, serum phosphate, serum sodium, serum potassium, serum magnesium, complete liver profile including serum bilirubin, serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST), alkaline phosphatase, parathyroid hormone level, hepatitis B surface antigen and 24-h urine protein measurement were recorded at baseline and then every 3 months thereafter. All patients were screened for HCV infection at the time of enrollment into the study and were classified into HCV-positive and HCV-negative groups. Assessment of HCV status was done by using enzyme-linked immunosorbent assay (ELISA) test followed by recombinant immunoblot assay in all patients. Those patients who were detected to be positive for either test underwent polymerase chain reaction (PCR) testing using an amplicor amplification kit (Roche diagnostics, Neuilly, France) according to the manufacturer’s instructions [20]. High-risk patients such as those who had received a blood transfusion, healthcare workers or other high-risk groups underwent PCR even when they were ELISA negative. The patients were followed up over a period of 6.7 ± 3 years, range of 1–14.4 years. The primary and secondary outcomes measures studied were death or the development of ESRD.

Treatment for LN

Both groups of patients received induction therapy with intravenous methylprednisolone 1 g daily for 3 days followed by oral prednisolone 1 mg/kg body weight which was progressively tapered to ~10 mg daily at the end of 6 months and intravenous cyclophosphamide 1 g/1.73 m² monthly for 6 months and then was replaced by oral azathioprine 2 mg/kg daily thereafter for a period of at least 2 years after the last relapse as per the standard hospital protocol, in addition to supportive therapy for BP and lipid abnormalities based on the physicians discretion. None of the HCV-positive patients were treated with antiviral therapy for HCV infection either with interferon alpha or ribavirin.

Statistical analysis

Cumulative probability of patients deteriorating to ESRD was estimated according to Kaplan–Meier Survival Analysis [21]. Comparison between the survival curves obtained from HCV-positive LN patients and HCV-negative LN patients was performed by means of log-rank test. The variables were age, gender, BP, baseline kidney function, serum creatinine, urinary protein and hepatitis status. Frequency analysis was used to grade the renal impairment. Descriptive statistics was calculated for quantitative variables. Means of the quantitative variables were compared by Student’s t-test taking P < 0.05 as the level of statistical significance. SPSS statistical package (SPSS Inc., Chicago, IL) version 12 was used for survival curves and statistical analysis.

Results

One hundred and thirty-four patients fulfilled the criteria for enrollment into the study. Fifteen (11.2%) patients were HCV positive of which 2 (13.3%) were male and 13 (86.7%) were female. One hundred and nineteen (88.8%) were HCV negative of which 17 (14.3%) were male and 102 (85.7%) were female. The mean age was 32.47 ± 11.8 years; 19 (14.2%) were male and 115 (85.8%) were female. Fifteen patients (11.2%) were HCV positive of which 2 (13.3%) were male and 13 (86.7%) were female. One hundred and nineteen (88.8%) were HCV negative of which 17 (14.3%) were male and 102 (85.7%) were female. There was no predilection for gender in the incidence of HCV infection. Thirteen percent of the males were positive for HCV infection in comparison to 14.3% of males who were negative for HCV infection. The probable reasons for the acquisition of the HCV infection in these patients were blood transfusions, dental procedures, sharing blades at the barber shops and bloodletting (blood shedding using a tool shared by many people for relieving high BP).

The mean age of patients in the HCV-positive group was 32.47 ± 11.8 years, while in the HCV-negative group it was 34.6 ± 12.3 without any statistical significance. Mean follow-up period was 6.7 ± 3.3 years with a range of 1–14.4 years. Fifteen patients were lost to follow-up in the HCV-negative group; however, none of the patients was lost to follow-up in the HCV-positive group. The baseline demographics and clinical characteristics of the LN patients are shown in Table 1. There was no statistically significant difference between mean systolic and diastolic BP between HCV-positive and HCV-negative patients. Systolic and diastolic BP was 140.1 ± 20.1 and 84 ± 11 mmHg in the HCV-positive patients and 145.2 ± 12.3 and 86.2 ± 7.4 mmHg in the HCV-negative patients, respectively, (P = 0.77 and 0.31, respectively). Nine (60%) patients were hypertensive with BP >130/90 mmHg HCV-positive group in comparison to 74 (62%) in HCV-negative group (P = 0.86). The mean 24-h urinary protein level was higher in the HCV-positive patients of 3.4 ± 3.8 g in comparison to HCV-negative patients of 2.24 ± 2.26 g; however, the values did not reach the statistical significance (P = 0.88), there was no difference in serum albumin levels between HCV-positive patients of 32.8 ± 8.4 g/L and negative patients 32.2 ± 7.1 g/L (P = 0.76) at the baseline. The blood urea and serum creatinine levels were 7.5 ± 5 mmol/L and 108 ± 53 μmol/L in HCV-positive patients and 7.5 ± 48 mmol/L and 103 ± 67.6 μmol/L in HCV-negative patients (P = 0.98 and 0.78, respectively). The mean total cholesterol in HCV-positive patients was 4.87 ± 1.9 mmol/L, while in HCV-negative patients it was 5.1 ± 1.3 mmol/L (P = 0.542). There was no statistically significant difference between AST and ALT levels between HCV positive and negative patients at the baseline with values of 20.2 ± 4.6 and 18.5 ± 4.7 IU/L for former and 18.2 ± 4 and 21.1 ± 3.9 IU/L for later with P = 0.07 and 0.18, respectively. We did not find any difference in the immunological profile like ANA and dsDNA levels between HCV positive and negative patients, the ANA and dsDNA levels in the former were 822.54 ± 908 and 598 ± 597.8, while in the latter, the levels were 1011.49 ± 1556 and 564.37 ± 705.4, respectively, with a P-value of 0.64 and 0.84, respectively. There was also no significant difference between fasting blood glucose (FBG) level in HCV positive and negative patients at the baseline with the FBG level in the HCV-positive patients being
5.62 ± 1.93, while as the FBG level in the HCV-negative patients being 5.86 ± 2.75 with a P-value of 0.15.

The baseline kidney biopsy of all the LN patients was evaluated for activity and chronicity index by the International Society of Nephrology renal pathological association (ISN-RPA) 2004 classification. The activity and chronicity index in HCV positive and negative LN patients was 3.26 (ISN-RPA) and 3.4 respectively, with a P-value of 0.63 and 0.21, respectively.

We did not find any difference in the usage of the angiotensin-converting enzyme inhibitors or angiotensin receptor blockers in the HCV-positive or the HCV-negative patients. There was also no difference in the cumulative dosage of cyclophosphamide or prednisolone received by the two groups, Figure 1.

We stratified our patients into three groups according to the serum creatinine levels, Group 1 serum creatinine <120 µmol/L, Group 2 serum creatinine 121–350 µmol/L and Group 3 serum creatinine >350 µmol/L. Of the 15 patients in the HCV-positive group, 5 (35%) were in Group 1, 2 (14.3%) were in Group 2 and 8 (53.3%) were in Group 3, while in the HCV-negative group of 119 patients, 33 (28.7%) were in Group 1, 32 (27.1%) were in Group 2 and 54 (45.7%) were in Group 3. The P-value was highly significant (P = 0.02) in Group 3 between the HCV positive and the negative patients at the last follow-up, Figures 2 and 3.

There was no statistically significant difference between serum creatinine levels at enrollment in HCV-positive and HCV-negative patients with values of 108 ± 53 and 103 ± 67.6 µmol/L, respectively, with a P-value of 0.783; however, the serum creatinine levels were significantly higher in the HCV positive 523.2 ± 290 µmol/L than in the HCV negative 260.7 ± 24 µmol/L patients at the end of the study (P < 0.001). Similarly, there was no difference between glomerular filtration rate (GFR) at enrollment between the two groups 59.2 ± 3.8 ml/min in HCV-positive patients and 58.6 ± 3.02 ml/min in HCV-negative patients (P = 0.943); however, the GFR was lower in HCV-positive patients 25 ± 34.9 ml/min than HCV-negative patients 43.3 ± 33 ml/min at last follow-up with a statistically significant P-value of 0.046.

There was no statistically significant difference in the FBG levels between the HCV positive and the HCV negative groups at the end of the study, the FBG level in HCV-positive patients was 6.8 ± 4.1 mmol/L and in HCV-negative patients was 5.9 ± 3 mmol/L with a P-value of 0.29.
**Fig. 2.** Renal function in patients with LN with HCV positive and negative at last follow-up. There was highly significant statistical difference between the Hep C positive and Hep C negative patients at the last follow-up with a P-value of $<0.001$.

**Fig. 3.** Creatinine clearance in patients with LN and HCV positive and negative at enrollment and end of the study. There was highly significant statistical difference between the Hep C positive and Hep C negative patients at the last follow-up with a P-value of $<0.001$.

**Table 2.** Renal outcome in patients with LN patients with HCV infection at the end of the study

<table>
<thead>
<tr>
<th>Parameters</th>
<th>HCV positive, $N = 15$ (%)</th>
<th>HCV negative, $N = 104$ (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBS (mmol/L)</td>
<td>$6.8 \pm 4.1$</td>
<td>$5.9 \pm 3$</td>
<td>0.29</td>
</tr>
<tr>
<td>ANA (mean ± SD)</td>
<td>$1467.8 \pm 1303$</td>
<td>$930.7 \pm 1578$</td>
<td>0.20</td>
</tr>
<tr>
<td>dsDNA (mean ± SD)</td>
<td>$331.524 \pm 532$</td>
<td>$334.94 \pm 551$</td>
<td>0.98</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>$62.8 \pm 13$</td>
<td>$21.6 \pm 4$</td>
<td>0.005</td>
</tr>
<tr>
<td>C3 g/L</td>
<td>$0.8 \pm 0.1$</td>
<td>$1.2 \pm 0.1$</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PT</td>
<td>$15.0 \pm 1.4$</td>
<td>$12.5 \pm 1.17$</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum albumin (g)</td>
<td>$26.6 \pm 8.2$</td>
<td>$36.3 \pm 5.3$</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Death</td>
<td>5 (33.3%)</td>
<td>8 (7.6%)</td>
<td>0.03</td>
</tr>
<tr>
<td>ESRD</td>
<td>8 (53%)</td>
<td>18 (17.3%)</td>
<td>0.005</td>
</tr>
</tbody>
</table>

We did not find any difference in the immunological markers like ANA and dsDNA levels between HCV positive and negative patients with a ANA and dsDNA level of 1467.8 ± 1303 and 331.524 ± 532 in HCV positive patients and 930.7 ± 1578 and 334.94 ± 551 in HCV negative patients, respectively, with a P-value of 0.20 and 0.98, respectively, however, we noticed that the C3 levels were significantly lower in HCV positive patients $0.8 \pm 0.1$ g/L than HCV-negative patients $1.2 \pm 0.1$ g/L with a P-value of $<0.001$. We also found a worsening of the markers of liver function in the HCV-positive patients than HCV-negative patients at the end of the study. The ALT, serum albumin and the PT levels were $62.8 \pm 13$ IU/L, $26.6 \pm 8.2$ g/L and $15.0 \pm 1.4$, respectively, in HCV-positive patients, while as ALT, serum albumin and the PT levels were $21.6 \pm 4$ IU/L, $36.3 \pm 5.3$ g/L and $12.5 \pm 1.17$ in HCV-negative patients, respectively, with a P-value of 0.005, 0.001 and 0.001, respectively.

Five (33.3%) HCV-positive LN patients died in comparison to eight (6.7%) HCV-negative LN patients with a relatively significant P-value of 0.03. Kaplan–Meier survival curves for HCV-positive verses HCV-negative patients are presented in Figure. HCV-positive patients had a rapid decline renal function leading to ESRD as compared to HCV-negative patients the difference was statistically significant (P < 0.05 log-rank test). The renal survival i.e. dialysis free at the end of 5 and 10 years was 95 and 80% for the HCV-negative group and 90 and 65% for the HCV-positive group with a highly significant P-value at the end of 10 years (P < 0.05).

**Discussion**

Chronic HCV infection is a global health problem with a prevalence of 0.9–18.1% in the general population all over the world; however, the prevalence is higher in a subset of patients on chronic HD ranging from 5 to 60%[9, 10]. Chronic HCV infection is one of the important risk factors for the development and progression to CKD. The prevalence of both chronic HCV infection and LN is quite high in Saudi Arabia, the prevalence of the former has been reported to be around 1.8% in Saudi Arabia [22], while as incidence of LN has been reported to be 47.9% by Al-Arfaj et al. [22]. In the present study, we report an incidence of chronic HCV infection of 11.2% in our Class IV LN patients. Since LN predominantly affects females more than males, it is expected that majority of our patients 86% were females; however, we did not find any predilection of HCV infection for either gender.

Chronic HCV infection is one of the most important risk factor for the long-term deterioration of kidney function, reduced graft survival and patient survival and is associated with increased incidence of renal allograft rejection [12, 22–24]. We stratified the LN patients depending upon the severity of the renal failure, majority of our HCV-positive patients in comparison to HCV-negative patients had serum creatinine $>350$ μmol/L at the end of the follow-up 53.3% versus 22.6%, respectively (P < 0.02); similarly, the serum creatinine levels and the creatinine clearance were higher and lower, respectively, at the end of the study in HCV-positive patients which were statistically significant. The renal diseases associated with chronic HCV infection include cryoglobulinemia, membranoproliferative glomerulonephritis and membranous nephropathy and IgA nephropathy whether the deterioration of kidney function is due to the superadded renal lesion attributed to HCV infection cannot be ascertained since we did not have repeat kidney biopsy.
available in these patients and also the cryoglobulin levels were not regularly monitored, we are not sure of contributions of cryoglobulinemia in the worsening of renal function in these patients. Other possible reason we could speculate for the more severe deterioration of renal function in the HCV-infected patients may be due to the lesser immunosuppressive medication exposure in the HCV-positive patients fearing the flare of the HCV infection; however, we did not find any difference in the dose and the type of the immunosuppressive medication exposure between the two groups, both groups received the hospital protocol-induction therapy with intravenous methyl prednisolone and intravenous cyclophosphamide followed by maintenance therapy with steroids and the azathioprine.

In a recent retrospective cohort study involving >470,000 adult veterans, patients with HCV infection were more likely to develop ESRD (4.3 per 1000 person-year) than HCV-seronegative patients (3.1 per 1000 person-year) [25]. Moreover, in patients with an estimated GFR 30 mL/min/1.73m², the presence of HCV was associated with a nearly 3-fold higher risk of ESRD. These findings were confirmed by a subsequent cross-sectional study showing that HCV-positive patients had a 40% higher likelihood for developing renal insufficiency compared with seronegative subjects [26]. Beside the risk of renal disease progression, the overall prognosis for patients with HCV-related nephritis is poor because of a high incidence of coinfections and CVD [27]. We observed, in our study, that more patients in the HCV group died than in HCV-negative group; however, none of these patients died of liver failure or cirrhosis, although there was significant deterioration in liver function tests as evidenced by the elevation in the ALT and prothrombin time (PT) and decrease in the serum albumin level in the HCV-positive patients at the last follow-up, suggesting that severe immunosuppression related to steroids and cyclophosphamide may have a role in flaring up the proliferation of the virus in these patients and exacerbating liver damage. We also noticed that the HCV-positive patients had significantly lower C3 levels in comparison with the HCV-negative patients at the last follow-up suggesting that these patients may have persistent immunological activity related to the LN or even low C3 may have been a consequence of HCV-related cryoglobulinemia which unfortunately was not evaluated in our study. At this point, it is very difficult to hypothesise whether the immunosuppression causes further viral proliferation which in turn leads to deterioration of the liver function and exacerbation of the lupus activity and hence leads to smoldering kidney damage. We suggest further studies should be carried out to unravel any still
unanswered questions regarding the special relationship between HCV infection and the kidney in general but particularly LN and the outcome in these patients which our study could not do due to many limitations we have encountered.

Limitations

We are still unsure of the cause for the deterioration in renal function in our patients although we can speculate a few culprits. We think it would have been quite informative if a repeat kidney biopsy was available during the course of the follow-up in these patients with deteriorating renal function to evaluate whether these renal lesions are related to underlying LN or some other causes as mentioned above, particularly immunofixation of the kidney sample for the HCV antigens would have been very valuable. We also believe that these patients should have been regularly monitored for cryoglobulinemia and the HBA1c levels both of these tests were not, unfortunately, done in these patients. Another important limitation of our study was that the follow-up quantitative HCV PCR was not, done which would have been very useful to assess the effect of the immunosuppression on viral proliferation in these patients.

Conclusion

The present study highlights that concomitant HCV infection in patients with underlying LN is associated with worse renal outcome, higher rate of progression to ESRD and reduced patient survival and liver damage. There is no gender predilection for HCV infection in patients with underlying LN. More intensive efforts should be made in the prevention and appropriate treatment of chronic HCV infection in patients with LN.

Conflict of interest statement. None declared.

References


Received for publication: 19.10.10; Accepted in revised form: 9.5.11