ABSTRACT

Purpose. Recurrence of HCV after LDLT is almost universal. Different factors affect response to treatment. Few data are available regarding outcome of recurrent HCV genotype 4. The purpose of this study is to improve outcome of recurrent HCV genotype 4 after LDLT.

Methods. An IRB approved chart review of 243 patients transplanted for ESLD, HCV genotype 4 over 4 years were reviewed. Protocol liver biopsies were taken 6 months after transplant. Patients received pegylated interferon and ribavirin in case of histological recurrence. Five patients had FCH were excluded.

Results. Thirty-seven patients were included. Sustained Virological Response (SVR) was achieved in 29 (78.3%). Patients with Metavir fibrosis stage (F0) and (F1) had SVR in 5/5 (100%) and 20/24 (83.3%). Two patients with F1 had to stop treatment because of thrombocytopenia and 2 were non responders. Three out of 6 patients (50%) with (F2) had SVR, 2 were non responders and one had to discontinue treatment because of severe depression. One of 2 patients (50%) with F3 had SVR and the other patient decompenesated within 4 months before treatment and died.

Conclusion. Protocol biopsies allow early detection of inflammatory changes in the graft before fibrosis occurs. Early treatment of recurrent HCV genotype 4 after LDLT results in better response.

HEPATITIS C VIRUS-induced liver failure is the leading indication for liver transplantation (OLT) in Egypt. Hepatitis C virus (HCV) recurrence is universal after OLT [1]. Lessons from treating the pretransplant population still apply to patients with recurrent post-transplant HCV while studies specific to this population proceed. The majority of HCV patients have minimal or nonprogressive liver injury after OLT and good long-term survival. In a subgroup of patients, however, a dramatic course of reinfection is associated with poor outcomes and is characterized by the development of either liver cirrhosis or cholestatic hepatitis [2,3]. Cholestatic hepatitis is a life-threatening recurrent pattern of HCV in immunosuppressed patients, for which curative treatment has not yet been established. Cirrhosis due to recurrent HCV occurs in at least 25% of patients within 5 to 10 years after OLT, and once cirrhosis has developed, there is an annual risk of decompensation of 42% [4].

Several studies have demonstrated a worse long-term outcome for HCV-positive compared to non-HCV patients in the post-transplant setting [5]. Results of retransplantation are generally or evenly poor [6]. Meanwhile, indications for treatment after transplant, the optimal timing, dose, and duration of treatment for patients with recurrent HCV infection post-transplant are still not standardized. Therapy may be initiated preemptively or early before the development of histologic and biochemical recurrent hepatitis, or may be started once recurrent clinical disease is evident. Early treatment would seem attractive because treatment is begun while viral levels are low and before the graft is damaged and theoretically may lead to higher sustained virologic response (SVR) rates. The relationship between the viral load and both the severity of HCV recurrence and long-term patient outcomes after OLT have been contradictory [7–9].

Few data are available regarding outcome of recurrent HCV genotype 4 after living donor liver transplantation (LDLT). Genotype 4 is predominant in Egypt [10,11]. Therefore, the aim of this work is to point out factors that
could improve outcome of recurrent HCV genotype 4 after LDLT in Egyptian cohort.

PATIENTS AND METHODS

Study Population
A total of 267 LDLT were performed between 2007 and 2012. An Institutional Review Board approved this chart review of 243 (92%) patients transplanted for end-stage liver disease due to HCV. All patients were checked for HCV genotype. All patients had HCV genotype 4. Protocol sonar-guided liver biopsies were taken 6 months after transplant by expert radiologists using tru-cut needles. Histopathologic revision of liver biopsies was done independently by 2 expert transplant pathologists. Sections of formalin-fixed, paraffin-embedded tissue were stained with hematoxylin-eosin-saffron, chromotrope, and Perl’s reaction. Metavir score was used to detect degree of inflammation and stage of fibrosis. Patients with fibrosing cholestatic hepatitis were also included. Follow-up liver biopsies were taken at 3, 6, and 12 months during treatment for early detection of immune mediated rejection induced by interferon. In case of mild biopsy-proven rejection, immunosuppressant therapy was adjusted. Biochemical response was identified by normalization of transaminases. Virologic response was identified by SVR. HCV RNA was measured quantitatively at time of transplant; before start of treatment; 3, 6, and 12 months after start of treatment; and 6 months after end of treatment using semi-automated reverse-transcription polymerase chain reaction (Dynamic range 12–1,000,000 IU/mL). Histologic response was identified by resolving hepatitis or stationary pathology compared to pathology at start of treatment in case of lack of virologic response. The study was performed in accordance with the ethical guidelines of the 1975 Declaration of Helsinki. Informed consent was obtained from all patients included in the study.

Antiviral Therapy
Patients received treatment in the form of combined pegylated interferon (PEG-IFN) α2a (180 µg subcutaneously per week) or PEG-IFN α2b (1.5 µg/kg/wk) and ribavirin in case of evidence of inflammatory histologic recurrence with or without any fibrosis. Our objective was to study correlation between stage of fibrosis and SVR. Ribavirin dose was adjusted between 400 and 800 mg/d according to level of hemoglobin. Erythropoietin was used for correction of anemia in case of hemoglobin level below 10 g/dL. Blood transfusion was needed in case of drop of hemoglobin below 8 g/dL despite erythropoietin use. Granulocyte colony stimulating factor was used in case of drop of total leukocyte count below 2000, or absolute neutrophil count below 750. Thrombopoietin receptor agonist was used when available in case of platelet count below 50,000.

Immunosuppression
In all patients, the initial immunosuppressive regimen was based on a calcineurin inhibitor, either cyclosporine or tacrolimus. In addition, patients received 500 mg of intravenous methylprednisolone after reperfusion. Starting on postoperative day 1, methylprednisolone was tapered from 200 to 20 mg within 5 days; thereafter, methylprednisolone was maintained at 20 mg/d and then tapered by 5 mg/mo and stopped at 3 months post-transplant. Tacrolimus level was kept between 6 and 8 ng/mL, and cyclosporine at 150 to 200 ng/mL during the course of treatment. Patients had to change to everolimus (3–8 ng/mL) in case of renal impairment with reduction of dose of tacrolimus to target level (3–5) or cyclosporine (100–150). Mycophenolate mofetil was used as part of initial triple immunosuppressive therapy or as maintenance immunosuppressive. Basiliximab was used for induction in patients with glomerular filtration rate <60 before transplantation.

Statistical Analysis
Categorical variables were expressed as percentages and compared with the χ² test. Continuous variables were presented as mean and standard deviation (range: minimum and maximum). The Kaplan-Meier method was used to estimate overall survival. The log-rank test was performed to evaluate survival differences between specific groups of patients. A Cox regression model was used to analyze the independent effects of different variables on survival. A P value <.05 was considered statistically significant. The calculations were performed with SPSS 17.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Patients Characteristics
Fifty-six (23%) had evidence of histopathologic recurrence of HCV. Forty-two patients finished the treatment. Five patients with fibrosing cholestatic hepatitis were excluded, and 37 patients were included in the study (Table 1). Recipient mean age was 41.6 ± 10.9 (range 31–60 years) and mean age for donors was 27.1 ± 4.2 (range 18–34 years). All patients received tacrolimus or cyclosporine with or without mycophenolate mofetil or mammalian target of rapamycin. Nineteen patients received PEG-IFN α 2a and 18 patients received PEG-IFN α 2b for 48 weeks.

Correlation Between Virologic Response and Fibrosis Stage
SVR was achieved in 29 (78.3%) out of 37 patients (Fig 1). Patients with Metavir fibrosis stage F0 and F1 had SVR in 5/5 (100%) and 20/24 (83.3%), respectively. Two patients with F1 had to stop treatment because of severe renal impairment with reduction of filtration rate <60. Only 1 patient had mild acute cellular rejection during treated proved with follow-up biopsy and improved with increasing dose of cyclosporine with target level of 200 ng/mL with mycophenolate mofetil 1 g twice daily. None of patients developed moderate or severe rejection.

Patient Survival
Our treatment protocol was associated with 5-year survival of 84% (Fig 2). There was a difference regarding survival of patients with no fibrosis or F1 compared with those with stages F2 or F3 (Fig 3). Nevertheless, this difference was not statistically significant (P = .65).

Hematologic Side Effects
Anemia occurred in 27 of 37 patients (73%). 22 patients required erythropoietin biweekly when hemoglobin dropped
below 10 g/dL, and 5 patients required erythropoietin 3 times per week with blood transfusion when hemoglobin dropped below 8 g/dL. All patients who started to develop anemia required erythropoietin until end of treatment. Neutropenia occurred in 17 patients (46%) and improved with granulocyte colony-stimulating factor. Thrombocytopenia occurred in 4 patients (11%), 2 improved on thrombopoietin receptor agonist, and 2 had to stop treatment. We tried as much as we can to continue PEG-IFN treatment weekly with close follow-up and immediate correction of hematologic side effects.

**DISCUSSION**

There are few published data about hepatitis C virus genotype 4. The Egyptian Demographic and Health Survey

**Table 1. Patients and Donors Variables Did Not Show Any Significant Effect on SVR**

<table>
<thead>
<tr>
<th></th>
<th>n (%)</th>
<th>Mean ± SD</th>
<th>Range</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recipient age (y)</td>
<td>41.6 ± 10.9</td>
<td>31–60</td>
<td>.6</td>
<td></td>
</tr>
<tr>
<td>Recipient sex</td>
<td></td>
<td></td>
<td></td>
<td>.89</td>
</tr>
<tr>
<td>Male</td>
<td>31 (84)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>6 (16)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood group</td>
<td></td>
<td></td>
<td>.48</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>16 (43)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>11 (30)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AB</td>
<td>1 (2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O</td>
<td>9 (25)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass index</td>
<td>27.8 ± 6.1</td>
<td>20–35</td>
<td>.47</td>
<td></td>
</tr>
<tr>
<td>Child Pugh score</td>
<td></td>
<td></td>
<td>.22</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>8 (22)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>29 (78)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCVPCR (IU/mL) before LDLT</td>
<td>1,310,783 ± 7,091,251</td>
<td>617–45,000,000</td>
<td>.4</td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>22 (59.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>12 (32.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>3 (8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCVPCR (IU/mL) before start of treatment</td>
<td>1,611,590 ± 6,685,875</td>
<td>327–33,000,000</td>
<td>.1</td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>2 (5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>8 (22)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>27 (73)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Donor age (y)</td>
<td>27.1 ± 4.2</td>
<td>18–34</td>
<td>.56</td>
<td></td>
</tr>
<tr>
<td>Donor sex</td>
<td></td>
<td></td>
<td>.67</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>29 (78)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>8 (22)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Steatosis in graft before LDLT</td>
<td></td>
<td></td>
<td>.78</td>
<td></td>
</tr>
<tr>
<td>No steatosis</td>
<td>33 (89)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5%</td>
<td>2 (5.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10%</td>
<td>2 (5.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type of interferon</td>
<td></td>
<td></td>
<td>.9</td>
<td></td>
</tr>
<tr>
<td>Pegylated IFN 2a</td>
<td>19 (51)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pegylated IFN 2b</td>
<td>19 (49)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immunosuppressive therapy</td>
<td></td>
<td></td>
<td>.64</td>
<td></td>
</tr>
<tr>
<td>Tacrolimus</td>
<td>17 (46)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tacrolimus + MMF</td>
<td>1 (2.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tacrolimus + MTOR</td>
<td>2 (5.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclosporine</td>
<td>15 (40.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclosporine + MMF</td>
<td>2 (5.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: SVR, sustained virologic response; SD, standard deviation; MELD, Model for End-stage Liver Disease; HCVPCR, hepatitis C virus polymerase chain reaction; LDLT, living donor liver transplantation; IFN, interferon; MMF, mycophenolate mofetil; MTOR, mammalian target of rapamycin.

![Algorithmic design for patients according to degree of fibrosis and their response rate. F0, F1, F2, F3, Metavir fibrosis stage; SVR, sustained virologic response; NR, nonresponder; D/C AE, discontinue due to adverse events.](image-url)
reported overall prevalence of HCV antibody 17.4% in males and 12.2% in females [12]. Most of these data are concerned with chronic hepatitis patients before OLT. Genotype 4 predominates in Egypt. There are a number of reports, and a study of 131 hepatocellular carcinoma and chronic hepatitis C patients found the following genotype distribution: 1 (6%), 3 (1%), and 4 (93% with 4a = 63%) [13,14]. Genotype 4 was found in Middle Eastern countries such as Egypt, Saudi Arabia, and Syria [15]. Some of these studies assumed that all Egyptian patients who are HCV positive have genotype 4. We tested all patients included in this study for HCV genotype and they all had genotype 4.

The main indication for OLT in Egyptian patients is HCV-related end-stage liver disease or hepatocellular carcinoma. And as long as recurrence of HCV after LDLT is universal, we thought that there should be a way to improve graft and patient outcome. A lot of studies referred to the predictors of recurrence and response to treatment after OLT for HCV but there are not enough data regarding how to improve outcome of recurrent HCV genotype 4 after OLT.

Controlled randomized and nonrandomized clinical trials demonstrated high SVR rates ranging between 50% and 79% in chronic HCV-4 hepatitis patients receiving PEG-IFN-a2b plus ribavirin before OLT [16–28]. Nevertheless, only few studies are available regarding the treatment of recurrent HCV genotype 4 after OLT with very small number of patients, and some patients were treated with conventional interferon in these studies [29–31]. To the best of our knowledge, our study includes the largest number of recurrent HCV genotype 4 recipients receiving PEG-IFN and ribavirin after LDLT.

In absence of deceased donors, we try to achieve the best survival and outcome for these patients because there is no possibility for retransplantation in case of graft failure. Also, OLT is financially demanding, which is why every effort should be done to maintain the function of the graft and guard against HCV recurrence and fibrosis progression. Looking at the strategies that have been proposed to improve the results of OLT in HCV-infected patients, we found that the eradication of HCV infection before transplantation with use of antiviral therapy was not applicable [32–35]. So the eradication of HCV infection immediately after transplantation to prevent graft damage [36–39] and the treatment of established recurrent hepatitis C in the acute or, more commonly, chronic phase were the alternatives [40–59]. As long as the response rate to treatment is higher in persons with acute than persons with chronic HCV infection, with higher SVR rate [60], we adopted our protocol of early treatment within 6 months after LDLT. We also performed liver biopsy for all patients at 6 months after transplantation to detect early fibrosis stages and any necroinflammation and to treat these patients early to improve SVR rate and improve overall survival of HCV-infected recipients. Previous studies also suggested better response rate and better survival in patients with low fibrosis stages [48,52]. Prolonged treatment could be beneficial to achieve histologic, biochemical, and virologic response for those who did not respond for 48 weeks of treatment rather than stopping treatment [57,61].

Though all of our donors were not old, our patients developed variable stages of fibrosis early within 1 year, so other factors like organ regeneration or viral load may also play a role in fibrosis progression and not only donor age as previously studied [62]. The SVR in our patients group was 78% compared to what previously reported to be only 30% (range, 8%–50%) in recurrent HCV genotype 1 after OLT [63]. This is attributed to our early treatment protocol, early detection of necroinflammation, treatment at early stages of fibrosis, exclusion of patients with recurrent FCH, and young donor age group. Regular follow-up with biopsies
during treatment to detect interferon-induced rejection early helped us to avoid fear of rejection during treatment. Overall, the incidence of rejection was previously reported to be low, at about 6.4%, and ranged from 0% to 25% [63].

Common causes of treatment discontinuation including anemia, leukopenia, and thrombocytopenia were avoided using erythropoietin, blood transfusion, granulocyte colony-stimulating factor, and thrombopoietin receptor agonist. That is why only few patients had to discontinue treatment in our series due to hematologic side effects or unavoidable, uncontrollable neuropsychiatric conditions or poor tolerability. Despite the use of adjuvant therapy with growth factors, dose reductions were necessary in the majority of patients. We tried to reduce only ribavirin but we did not reduce PEG-IFN.

Our study was limited by the sample size that precluded an adjusted analysis to determine the relative importance of different factors that achieve a better SVR.

In conclusion, early treatment of recurrent HCV has better response rate. Protocol biopsy is important to detect early necroinflammatory changes and fibrosis.

ACKNOWLEDGMENT

We thank Dr Ahmad Abdelmakasoud for his help and support.

REFERENCES

OUTCOME OF RECURRENT HCV GENOTYPE 4 AFTER LDLT


