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Assessment of Hepatitis C Virus (HCV) recurrence impact on the patient and survival after liver transplantation.

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Abstract: Hepatitis C virus (HCV) related liver disease is the primary cause of cirrhosis and hepatocellular carcinoma (HCC) globally, leading to liver transplantation (LT). Although LT effectively reduces morbidity and mortality, patients with HCV-related end-stage liver disease inevitably face universal re-infection of the transplanted liver. When viral RNA is detectable during LT, HCV re-infection occurs, causing chronic hepatitis in at least 50% of grafts after one year and up to 100% after five years. This study aimed to identify HCV development risk factors and assess the consequences of HCV recurrence post-LT on patients and transplanted livers. The research involved 37 LDLT patients (18-65 years old) and 30 healthy individuals, measuring serum HCV-RNA and biochemical parameters. Results showed HCV recurrence in 32.4% and 45.9% after 3 and 6 months post-LT, respectively, compared to the control group. There was a significant difference in biochemical parameters between recurrence and non-recurrence patients. Despite achieving sustained virologic response (SVR) before LT, recurrent HCV becomes more pronounced three months post-LT, negatively impacting both graft and patient survival, with a substantial portion experiencing significant liver damage after LT.

keywords: Living donor, liver transplantation (LDLT), HCV infection, recurrence

Introduction

Liver disease causes two million deaths each year, accounting for 4% of all fatalities. The primary reasons for these deaths are complications arising from cirrhosis and hepatocellular carcinoma (HCC). Globally, viral hepatitis, alcohol consumption, and non-alcoholic fatty liver disease are the leading causes of cirrhosis [1].

LT has been accepted as a standard treatment providing the chance of survival for patients with acute liver failure or end –stage chronic liver diseases [2,3], and improvement in patients' life expectancy [4,5].

In Egypt, living donor liver transplant (LDLT) was first performed in Egypt in 1991. By the end of June 2016, the total number of cases reached 2,406. This number comprised 93% adult cases and rest is the pediatric cases (7%). The vast majority of indications were HCV hepatitis. [6]

For LDLT, graft failure outcomes were 6.1%, 8.7% in 6 months and 12 months respectively [7].

Liver transplantation impacts the health of patients [8].

LT enables transition from critical illness back to health [9,10].

The majority of individuals can resume work activities within 3 to 6 months following a transplant. Those who undergo LT have the capability to lead lives that are considered "normal" [11].

Recurrence of HCV was characterized by graft malfunction indicated through biochemical markers and the presence of detectable HCV RNA using a polymerase chain reaction assay. This was subsequently verified through histological confirmation as recurrent HCV [12].

The aim of our study is to provide insights into the factors influencing HCV recurrence

post-LDLT and its impact on the overall outcomes of liver transplantation.

Subjects and methods

Patients

Participants were recruited from liver transplantation unit, Gastro Intestinal Surgery Center, Faculty of Medicine, Mansoura University, from Januarys 2022 to September 2023. The samples were collected according to the ethical standards of Institutional Research Board (MS.22.09.2112), Faculty of Medicine, Mansoura University.

The study conducted on 37 subjects had LDLT for HCV infection and received antiviral therapy before LDLT and 30 apparently healthy volunteers are chosen as control. The inclusion criteria were recipients of LDLT, age > 18 year, maintained sustained virological response (SVR) 12 weeks. SVR as confirmed by negative HCV-RNA. Exclusion criteria were co-infection with other viruses. Blood samples were gathered from all subjects.

All included patients and control groups were subjected to the following:

1. Liver function test as liver transaminases (ALT), liver transferase (AST), international normalized ratio (INR), bilirubin (Bil), and alkaline phosphates (ALP) in serum was measured by Cobas C311 Fully automated clinical chemistry analyzer (Roche – HITACHI. Japan) (Roche Molecular Systems, Inc., Branchburg, NJ) after 3 and 6 months from LDLT.
2. Platelet count was determined through testing complete blood count while aspartate aminotransferase ratio index (APRI) score was calculated by using the formula, [(AST/ULN)/platelet counts (10^9 /L)] ×100 for every patient and control **[13].**
3. Detection of HCV RNA in LDLT patients by RT PCR technique after 3 and 6 months from LDLT with Cobas analyzer (CTM 48; Roche) according to
4 manufacture instruction. Lower limit of detection is 15 IU/ml. According to the HCV RNA results, patients were defined as SVR (HCV RNA negative 12 weeks after the end of therapy) or non responders.

Statistical analysis

SPSS software, version 23 (SPSS Inc., PASW statistics for Windows version 23), was used to analyze the data. SPSS Inc., Chicago. Number and percentage were used to describe qualitative data

Results

The study enrolled 37 patients with LDLT who received different sofosbuvir before transplantation and achieving SVR after 12 weeks of treatment. These patients were studied to detect HCV recurrence after LT. Characteristic of LT patients and healthy controls are shown in the current study. From Table (1), the study participants involve 37 patients (cases) had LDLT and 30 healthy individuals as a control group. Their age ranged from 18 to 65 years while the age of control group ranged from 21 to 48 years. The mean age of the patients was 45.6 $\pm$ 15.3 years, most of them (26/37) were men (70.3%). A significant was $p<0.001$ and $p = 0.03$ for age and gender of patients in comparison to control group (Table 1).

By comparing the data of liver profile, ALT, AST, INR, Bil, ALP and APRI score of patients and control group, there was a significant higher of these parameters in patients (3 and 6 months after LT) compared to control group ($p < 0.001$) as shown in Table 1. Similarly, liver enzyme levels for patients 3 months post LT were higher than that after 6 months (86.5 U/l vs 63.7 U/l, 84.5 U/l vs 60.4 U/l, 2.4 mg/dl vs 1.7 mg/dl in case of ALT, AST and Bil respectively while there was no difference in case of INR (1.4 vs 1.3) and ALP (8.2 vs 8.7).

After 3 months, HCV RNA was tested for patients. Table 2 regarding sever HCV recurrence was noted after 3 months post LT and showed a 32.4% (12/37) of the case had HCV recurrence while 25 cases was not, while this percentage was elevated to 45.9% after 6 months (Figure 1). The variation of the liver profiles after LT for the recurrence and non recurrence patients was clear, since the values of ALT, AST, and Bil were significantly differenced as 157.5 U/l Vs 52.4 U/l ($p<0.001$), 153.2 U/l vs 51.5 U/l ($p<0.001$), 2.1.vs 1.2 and 4.7 mg/dl vs 1.1mg/dl ($p=0.002$) for recurrence and non recurrence respectively, while there

was no difference in ALP and INR between both groups.

Rejection after LT was appeared in HCV recurrence patients through the first 3 months, since 4 cases (4/12= 33.3%) were rejected. On other hand, no rejection obtained through the next 3 months. This variation was appeared through calculation of APRI score which was 2.7% vs 0.88% (p<0.001) for recurrence and non recurrence respectively after 3 months (Table 2) (Figure 2).

Out of 12 HCV recurrence patients, four had acute cell rejection (33.3%), five (41.7%) were advanced fibrosis, 2 (16.7 %) were cirrhosis and only one case was intact liver architecture (8.3%). (Table 3) (Figure 3).

Table 1: Comparison of the clinical and biochemical measurements within the studied groups

parameters	After 3 month(n= 37)	After 6month(n=37)	Control(n=30)		*P*
Age (years)					
Mean ± SD	45.6 ± 15.3		34.33± 8.8		**< 0.001**
Gender M(%)F(%)	26 (70.3%)11(29.7%)		(46%)%)16(53.3%)		**0.031**
Biochemical characteristics					
ALT (U/l)					
Mean ± SD	86.5 ± 85	63.7 ± 49.7	21.7± 3.2		**< 0.001**
AST (U/l)					
Mean ± SD	84.5 ± 91.5	60.4 ± 40.7	21.6± 2.2		**< 0.001**
ALP					
Mean ± SD	8.2 ± 3.70	8.7 ± 4.9	5.5± 0.77		**< 0.001**
APRI					
Mean ± SD	1.5± 1.4	2.7 ± 8.1	0.25 ± 0.09		**< 0.001**
Bilirubin					
Mean ± SD	2.4 ± 1.6	1.74 ± 1.01	0.51± 0.11		**< 0.001**
INR					
Mean ± SD	1.38 ± 0.78	1.32 ± 0.66	1.02 ± 0.05		**< 0.001**
HCV recurrence Yes (%)No(%)	12(32.4%) 25(67.6%)	17(45.9%)20(54.1%)			-

ALT: alanine aminotransferase;**AST:** Aspartate aminotransferase;;**ALP:** Alkaline phosphatase**, APRI:** aspartate aminotransferase to platelet ratio index;**INR:** international normalized ratio. All data presented as mean ± SD, median and IQR (first quartile–third quartile) except sex and HCV recurrence presented as numbers**.** $p \leq 0.05$ is significant.

Table 2: Comparison of the clinical and biochemical measurements within the recurrence and non recurrence LDLT patients

parameters	HCV Recurrence (n= 12)	Non Recurrence (n=25)	Control (n=30)		*P*
Age (years)					
Mean ± SD	41.7 ± 15.3 47.3 ± 15.3		34.33± 8.8		**< 0.001**
Gender M (%)F (%)	4(33.3%) 6(24%) 8 (66.7%) 19(76%)		14(46.7%)16(53.3%)		**0.031**
Biochemical characteristics					
ALT (U/l)					
Mean ± SD	157.5 ± 111.7	52.4 ± 37.3	21.7± 3.2		**< 0.001**
AST (U/l)					
Mean ± SD	153.2 ± 127.2	51.5 ± 40.7	21.6± 2.2		**< 0.001**
ALP					
Mean ± SD	8.4 ± 2.6	8.1 ± 4.3	5.5± 0.7		**< 0.001**
APRI					
Mean ± SD	2.7± 1.9	0.88 ± 0.6	0.25 ± 0.1		**< 0.001**
Bilirubin					
Mean ± SD	4.7 ± 2.8	1.1 ± 0.7	0.50± 0.1		**< 0.001**
INR					
Mean ± SD	2.1 ± 0.7	1.28 ± 0.5	1.0 ± 0.05		**< 0.001**
Rejection 4 (41.7%)					

ALT: alanine aminotransferase;**AST:** Aspartate aminotransferase; **ALP:** Alkaline phosphatase**, APRI:** aspartate aminotransferase to platelet ratio index;**INR:** international normalized ratio. All data presented as mean ± SD, median and IQR (first quartile–third quartile) except sex and PCR presented as numbers**.** $p \leq 0.05$ is significant.

Table (3): Pathology results of the 12 HCV recurrence patients after 3 months LT.

Pathology (Recurrent)				
Rejection	**estimated cirrhosis**	**Adv fibrosis**	**Intact liv architecture**	**total**
4	2	5	**1**	12

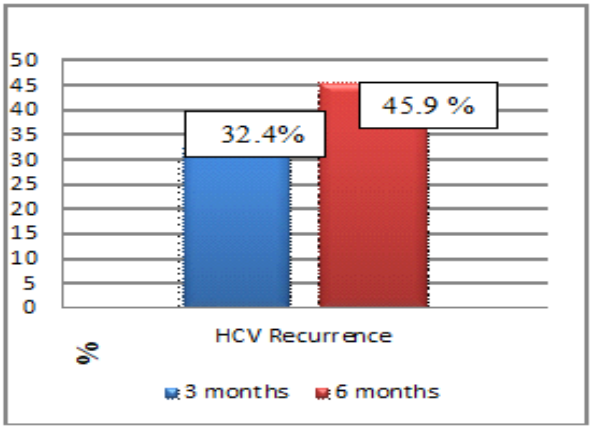

Figure (1): Percentage of HCV recurrence after 3 and 6 months LT.

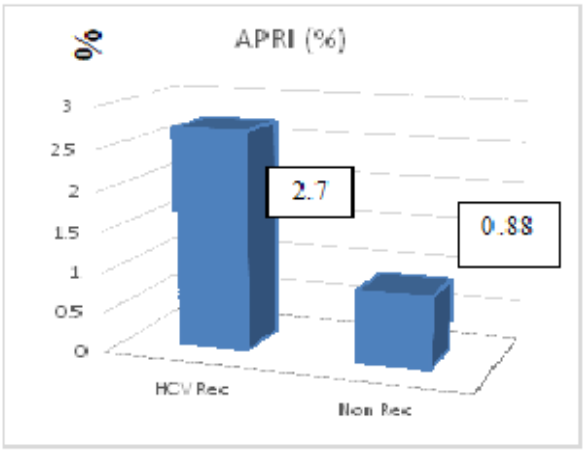

Figure (2): Mean APRI score for HCV recurrence and non recurrent patients after 3 months LT.

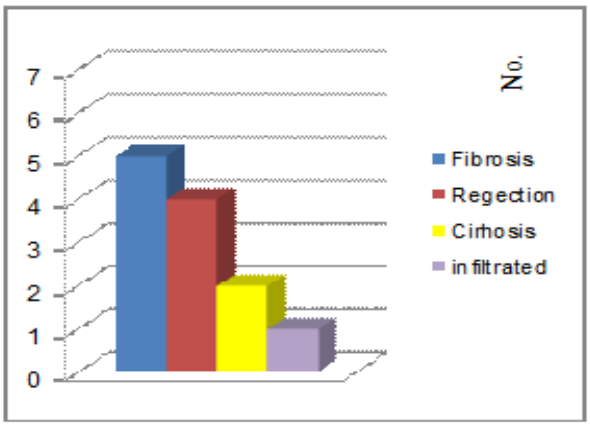

Fig (3): Subgroup of pathology of 12 HCV recurrence patients after LT.

Pathology of Recurrence patients

Discussion

HCV infection is the main indications for LT **[14,15]**, and for LDLT in Egypt **[6]**. Re-infection almost inevitable in patients with RNA undergoing LT **[16,17].**

In this study, patients who achieve a sustained virological response (SVR) underwent LT were examined for the presence of HCV-RNA recurrence 3 and 6 months after transplant to detect effect of HCV development, and impact of recurrence on patient and graft.

In our study, HCV recurrence was detected in 12 out of 37 (32.4%) after 3 months (12 weeks) LDLT while 25 were not, and it was detected in 17 out 37 (45.9 %) after 6 months. Our results are in accordance with many previous studies which represented a recurrence of HCV after LT as Argarwal et al., that included 79 patients with HCV recurrence after LT, use treatment associated with a 96% cure rate **[18]**. In another study, HCV-RNA found in 3.9% **[19]**. Kwok et al.,showed 204 patients were have HCV recurrence with SVR at 8, 12 weeks **[20]**.Also A study of Kumar et al., showed that sever HCV rscurrence was noted in 17% at 1 year after LT versus 30% at 3 years**[12].**

Reinfection is inevitable post-LT for HCV-related liver disease. The serum viral load reflects the interplay between viral production by infected cells and clearance by the host immune system. A rise in the serum viral load indicates established infection and new virus production by the infected allograft. Once the new liver is infected, hepatic viral replication resumes, leading to serum HCV RNA levels surpassing pre-LT levels **[21,22,23]**.

Without antiviral treatment, Hepatitis C Virus (HCV) infection recurrence post-Liver Transplant (LT) is almost inevitable and tends to advance, increasing the risk of graft loss **[24].** Various factors, such as HCV RNA levels at the time of transplantation, the age of donors, induction therapy choice, and cytomegalovirus (CMV) infection, are associated with the likelihood and progression of recurrent HCV after LT **[25,26,27]**.

HCV recurrence after LT will affect on both patients and graft and this was cleary obtained in our study on the liver profile which was

differed from the control group and also on the number of acute rejection cases which obtained. Serum levels of ALT, AST, and Bil was differing in recurrence group according to the non-recurrence one and in accordance to the control group also. It was higher in recurrence than that in case of non recurrence and control ($p< 0.001$), while there was no difference in values of INR and ALP between two groups ($p= 0.7$). This variation was appeared through calculation of APRI score which was 2.7% vs 0.88% for recurrence and non recurrence respectively after 3 months. APRI index predicts fibrosis and cirrhosis in hepatitis C patients and offers a non-invasive way to predict which patients have fibrosis and cirrhosis without imaging or biopsy **[28]**, and it measures how healthy liver is when you have a liver disease. So the lower the APRI score (<0.5), the greater the negative predictive value and ability to rule out fibrosis and cirrhosis, while the higher value (> 1.5) the greater the positive predictive value and obtain to rule in fibrosis **[13,28].**

Conclusion and recommendations

Recurrent hepatitis C, including severe recurrence, was greater 3 months following LT although patients achieve SVR before LT. Also, HCV recurrence has bad effect on both graft and patient survival. Since results of our study confirm that a significant proportion of patients with HCV recurrence developed significant liver damage after LT.

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