INTRODUCTION
Liver Transplantation (LT) is the current standard of care for End-Stage Liver Disease (ESLD), as a result of many congenital and acquired diseases. The two most common reasons of LT are cirrhosis and Hepatocellular Carcinoma (HCC). Causes of cirrhosis are multifactorial, however, hepatitis B (HBV), hepatitis C (HCV), and alcoholism are the most common causes. In past only cadaveric livers were utilized for LT. Now with advancements in surgical techniques, Live Donor Liver Transplant (LDLT) can be performed successfully in centers which have expertise in it.1,2

With longer survival of recipients, there is a broad spectrum of histopathological findings in the liver graft biopsies which must be carefully assessed. In many cases, there are more than one reasons leading to graft dysfunction.3 In such cases, percutaneous liver graft biopsy plays an important role to look for the predominant cause and hence aid in management.4,5

The commonly encountered findings in post-LT setting are broadly divided into three categories: (i) new-onset post LT abnormalities (early and late), (ii) acute rejection and (iii) recurrence of original disease. Banff schema 1997 of rejection activity index (RAI), modified histological activity index (mHAI) and recent literature were utilized for evaluation. The results were finalized in the light of clinical details along with relevant laboratory investigations and radiological findings.

RESULTS: Seventy eight percutaneous hepatic graft biopsies of 59 patients were evaluated. Among them, findings noticed in descending order of frequencies were Acute Cellular Rejection (ACR) in 37% (n=30), recurrent hepatitis C (HCV) in 22% (n=18), cholestasis/ductular proliferation in 27% (n=22), ischemic/reperfusion injury in 9.8% (n=8) and drug-induced liver injury in 3.7% (n=3). In the first six months post LT, ACR was the commonest cause of graft dysfunction, while recurrent HCV was noticed to be predominant reason after 6 months.

CONCLUSION: In this study, ACR was the most frequent finding in graft biopsies, followed by recurrent HCV. However, in first six months, ACR is the commonest histopathological finding while recurrent HCV was more frequently documented after 6 months.


ABSTRACT
Objective: To study the spectrum of histopathological findings in live donor liver graft biopsies.
Study Design: Case series.
Place and Duration of Study: Histopathology Department, Shifa International Hospital, Islamabad, from January 2011 to March 2014.
Methodology: The biopsies were received in formalin and routinely processed. The changes encountered were divided into three categories: (i) new-onset post liver transplantation (LT) complications (early and late), (ii) acute rejection and (iii) recurrence of original disease. Banff schema 1997 of rejection activity index (RAI), modified histological activity index (mHAI) and recent literature were utilized for evaluation. The results were finalized in the light of clinical details along with relevant laboratory investigations and radiological findings.

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METHODOLOGY

This was a retrospective observational study, carried out at Histopathology Department, SIH, Islamabad, after approval of institutional review board. All liver graft biopsies received, including in-house and abroad transplanted patients, irrespective of age and gender, from January 2011 to February 2014, were enrolled in the study. Non-probability, consecutive sampling technique was performed. Biopsies with features of ACR, recurrent hepatitis, cholestasis/ductular proliferation, ischemia/reperfusion injury and drug induced liver injury were included. The LT biopsies included in the study were those where the recipient had undergone LDLT for any reason, and biopsy at any time after LDLT, for deranged LFT or any abnormality in radiological investigations, and receiving immunosuppressive therapy i.e. cyclosporine (CyA) or tacrolimus (FK506).

Follow-up of previous biopsy, were included while biopsies of patients who were receiving antiviral therapy post LT and biopsies other then five parameters under study were excluded.

The biopsies were received in formalin and routinely processed. Multiple serial levels of hematoxylin and eosin (H&E), one to two levels of each special stain (DPAS, reticulin, perl, orcein and trichrome) and immunostain CK7 (DAKO) were performed on each graft biopsy. Histopathological assessment was performed in accordance to clinical data, including reason and duration of transplantation, immunosuppressive therapy, trend of LFT, hepatic serology/Polymerase Chain Reaction (PCR) and with radiological findings if any, to reach a meaningful diagnosis.

Five parameters i.e. ischemia/reperfusion, ACR, ductular proliferation/cholestasis, drug-induced liver injury and recurrent diseases were assessed. Internationally accepted Banff schema 1997,9 RAI and mHAI were used.6 Recently published criteria for other histopathological findings in liver graft biopsies was utilized.6,10

Statistical software, SPSS, version 17.0 was utilized for data analysis. Frequency and percentage were calculated for qualitative variables like gender, reason of transplant, period at which biopsy was performed and five studied findings in liver graft biopsies i.e. ischemia/reperfusion injury, ACR, ductular proliferation/cholestasis, drug-induced liver injury and recurrent diseases. Mean ± SD was calculated for quantitative variables like age.

RESULTS

Seventy eight percutaneous hepatic graft biopsies of 59 patients were evaluated, who had LDLT for ESLD, by and large related with HCV alone (n = 42, 52.5%). Other causes included were HCC with HCV in 11.5% (n = 9), cryptogenic cirrhosis in 5.1% (n = 4), HCV/HBV in 2.5% (n=2) while, HBV/Hepatitis D (HDV), autoimmune hepatitis, non-syndromic paucity of bile ducts, Alagille syndrome, drug/ischemia induced graft injury and suspected Wilson disease in 1.4% (n = 1). The cause was unknown in 1.4% (n = 1).

Nineteen patients had more than one biopsy performed during post LT period. The age range was 5 - 74 years, with mean age of 74 ± 14.6. Out of 59 patients, 40 (67.8%) were males and 19 (32.2%) were females. Among them, reasons of graft dysfunction noticed in descending order of frequencies were ACR in 39.2% (n=31), recurrent HCV in 22.8% (n=18), cholestasis/biliary proliferation in 11.4% (n=9), ischemic/reperfusion injury in 2.5% (n=2) and drug-induced liver injury in 1.3% (n=1). In few cases, there were multiple findings; two cases have both cholestasis and ACR; one had ACR, recurrent hepatitis C and vascular obstruction; one had ACR and recurrent hepatitis C. In one case, late cellular rejection and recurrent hepatitis C were found, while in 6 cases non-specific findings were noticed. Other findings noticed in few biopsies were suspicious of viral infection and early chronic rejection in one case each and acute cholangitis in 2 cases.

Post LT duration was arbitrarily divided into early period (0 - 30 days), mid period (31 - 180 days) and late period (more than 180 days) to identify the spectrum of various histopathological findings at different intervals. These included 29.1% (n = 23) biopsies in early period, 20.3% (n = 16) in mid period, 35.4% (n = 28) in late period, while in 13.9% (n = 11) duration post LT was unknown. During the first 6 months post LT, ACR (n=21), was the commonest cause of graft dysfunction, while recurrent ischemia/reperfusion, ACR, ductular proliferation/cholestasis, drug-induced liver injury and recurrent diseases were assessed. Internationally accepted Banff schema 1997,9 RAI and mHAI were used.6 Recently published criteria for other histopathological findings in liver graft biopsies was utilized.6,10

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**Table I:** Frequency of histopathological findings in liver graft biopsy at different periods after live donor liver transplantation.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>&lt; 1 Month</th>
<th>1 - 6 Months</th>
<th>&gt; 6 Months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute cellular rejection</td>
<td>11</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>Re-perfusion / Ischemia injury</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cholestasis / Bile duct proliferation</td>
<td>5</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Recurrence of HCV</td>
<td>1</td>
<td>1</td>
<td>13</td>
</tr>
</tbody>
</table>

Figure 1 (a,b): Moderate acute cellular rejection. This patient presented about 5 months and 20 days after LDLT with deranged LFT. Biopsy showed portal areas expanded by dense mixed inflammatory infiltrate in portal area (including lymphocytes, eosinophils), endotheliitis and bile duct inflammation (ductulitis). RAI was scored as 6 out of 9. Magnification (a) 20X and (b) 40X.
HCV (n=13) was noticed to be the predominant reason after 6 months (Table 1). Histological features of ACR and recurrence HCV are depicted in Figure 1 and 2.

DISCUSSION

National Institutes of Health confirmed LT, as a mode of therapy for ESLD patients in 1983. In the beginning, only cadaveric livers were utilized, but now with advancements in surgical techniques including perioperative care and increasing number of liver graft candidates, LDLT was initiated. The overall graft survival has improved remarkably for 1st year of 85 - 87% and for 5 years of 72 - 73%.2,11

In broad terms, indications of LT include ESLD, acute liver failure, and HCC. The most prevalent reason of ESLD in third world countries is HCV related cirrhosis. Other less frequent reasons of LT include failed liver graft and metabolic diseases.11

Liver graft pathology is a rapidly growing field and post LT setting has been complex from its initiation. It has now become even more challenging due to improvement in long-term graft outcome and prolong survival of recipients.7,12 Graft dysfunction can occur due to a number of reasons, ranging from operative complications, rejection, drug toxicity and recurrence of original disease. Timely and accurate diagnosis is extremely crucial and aids hepatologists in appropriate patient management.13 In the past, ischemic/reperfusion injury, ACR, surgical and postoperative complications were more common reasons of graft dysfunction.13,14 The ACR was first time observed by Snover as a diagnostic triad: portal inflammation, bile duct damage, and endothelitis, is a common complication.15 In addition, recurrent hepatitis particularly in combination with ACR poses a diagnostic challenge for pathologists.13 Comparison between previous and follow-up biopsies should also be carried out, whenever possible for appropriate diagnosis.5

Post LT biopsies show following complications in decreasing order of frequency. Keeping in view the duration post LT, in early period, preservation injury and non-immunologic injury to the graft during harvesting and implantation are most prevalent. Preservation/reperfusion injury is manifested as parenchymal changes like centrilobular inflammation, cholestasis, hepatocytes ballooning, steatosis and rarely had significant graft dysfunction. These features had shown no correlation with histological features related to ACR.12,16 Centrilobular necrosis/centrilobular hepatocyte loss should raise suspicion of rejection, even in the absence of typical portal tract changes, and augmentation of immunosuppression should be considered.16

Ischemic complications, like hepatic artery thrombosis, are even more serious early complications and may lead to early graft loss or biliary stricture.17 Cholestasis in liver biopsy is one of the features of medicinal and chemical agents related liver injury. However, there are no specific lesions for drug-induced liver injury. The diagnosis must closely correlate with clinical history and after ruling out other possible complications.18

Ischemic-reperfusion injury and ACR were the most common early post LT complications.7 It was much higher in an older study by Yu et al., reaching up to 48.2%.23 In this series, there were 31 cases of ACR (39.2%). Geramizadeh et al. reported 44.4% rejection.21 Reperfusion/ischemic injury was 2.5% (n=2) in these cases.

In a review of 2004-2013 literature, the perfusion/ischemic injury documentation showed most variability. Shin et al. had reported it alone in 3.7% and 9.1% in combination with recurrent disease.7 Geramizadeh et al., Guo et al. and Cong et al. reported it in 7%, 9.7% and 13.4% of the graft biopsies respectively.4,21,22 Reperfusion/ischemic injury has declined over time due to improvement in surgical techniques. The small number cases of reperfusion/ischemia injury points to good surgical technique, and is close to recently reported figures. However, ACR still remains a major problem in post LT setting, as is documented in our and other studies. The probable reason is immune response of recipient against implanted graft and inadequate immunosuppression, due to different reasons, like non-compliance.

In the present study, majority of patients had HCV related LT (66%), its recurrence had a major proportion of the results, that is 22.8% (n=18). While in the studies of Geramizadeh et al., Guo et al. and Shin et al., HCV made a small proportion of recurrent diseases. So, as expected, Geramizadeh et al., Cong et al., Yu et al. and Shin et al., reported recurrence of hepatitis in 3.4%, 5.6%, 7.4% and 17.1%, respectively.4,7,21-22
The results of biliary ductular proliferation/cholestasis were quite high in this study (11.4%), as close to results of Shin et al. (17.1%), which showed marked difference from findings of Geramizadeh et al. (3.4%) and Cong et al. 5.6%, 7,21,22 It occurs mostly due to result of biliary stricture, and in many cases is a post surgical complication. With improvement in surgical technique, the number of such cases decrease.

Drug-induced liver injury was suspected in only one of these cases (1.3%), which was quite close to results of Geramizadeh et al. (1.8%) and Cong et al. (5%). 21,22 While there is quite proportion of recipients having drug related injury, as narrated in studies of Yu et al. and Guo et al. i.e. 11.1% and 26.1%, respectively. 4, 22 It was quite high in older studies and is less than 5% in recent studies. It points to the fact that with experience in LDLT, this complication has decreased.

In this study, five biopsies had more than one diagnosis. Ray et al. had mentioned concomitant pathologies like viral infection, biliary flow obstruction and ischemic complications, in diagnosed ACR. 12 Shin et al. had discussed combined causes of late graft dysfunction in 8.9% of the biopsies, among them ACR and biliary complications made 40.9%, 7 As already discussed, post LT graft biopsy analysis is a complicated field and requires close clinicopathological correlation. In biopsies, one can encounter more than one findings and their identification is essential as management is different.

Recurrent HCV was the predominant reason of graft dysfunction in late period (> 6 months) in 46.4% (n=13), while ACR made 25% (n=7), of received biopsies. However, recurrent HCV had been reported as early as 2 months following LT, as documented by Ziarkiewicz-Wróblewska et al. 20 In Gallegos-Orozco et al. study, 206 patients underwent LT for HCV related cirrhosis. 24 The overall frequency of recurrent diseases were 2.6%, 6.7% and 19.1% in other studies, 4, 7, 21 and among these 30.5% of recipients presented with recurrence within 1 year of LT and ACR was present in 27%. 24 Difference in trend is probably due to variation in prevalence of HCV in different regions.

CONCLUSION

In liver graft biopsies, the most common cause of graft dysfunction reported during first 6 months was ACR, while recurrent HCV was predominant reason after 6 months.

REFERENCES


