

ORIGINAL ARTICLE

Donor biopsy in living donor liver transplantation: is it still relevant in a developing country?

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Abstract

Liver transplantation is an important modality of treatment for end-stage liver disease. Liver biopsy evaluation has been an important aspect of the donor evaluation protocol. With the advent of newer modalities of donor evaluation such as high resolution CT scan, fibroscan and NMR spectroscopy, the relevance of the liver biopsy appears to be diminishing. We investigated the usefulness of donor liver biopsy evaluation in patients who had been cleared by radiological investigations. We evaluated 184 donor liver biopsies performed over a one-year period and found that 18% showed >5% steatosis and around 40% showed portal inflammation, which was, however, minimal to mild. Fibrosis was detected in 10 cases (5.4%), 7 being in stage 1 and 3 in stage 2. Donors with these findings were not considered for transplantation. We conclude that the liver biopsy still continues to be relevant especially in a developing country and does add additional information to the diagnostic work-up of a liver donor.

Keywords: donor, liver biopsy, transplantation, steatosis

INTRODUCTION

Living donor liver transplantation (LDLT) is an important modality and the treatment of choice for end-stage chronic liver disease (CLD) in developing countries where obtaining a liver for deceased donor liver transplantation (DDLT) is difficult. Most transplantation centres have protocols for donor evaluation, which includes a multi-step process. Donor liver biopsy, although recognized to be an important modality of evaluation, is not taken up in all centres. One study reported that only 14% of 42 evaluated transplant centres, perform biopsies of all donor livers, while 60% performed it in selected cases and 26% did not perform it at all.¹ At our centre, liver biopsies are evaluated in selected cases where other screening modalities gave an abnormal result, or if the body mass index (BMI) of the donor is high (>28 kg/m²) or abnormal liver enzyme levels are identified. The various factors considered to indicate a prospective donor liver biopsy are: history of alcohol intake, elevated serum ferritin levels and presence of steatosis on imaging.² There are many upcoming modalities like NMR spectroscopy for metabolic assessment

of the liver.³ We set out to identify the significance of donor liver biopsy especially in a developing country where liver biopsy is much cheaper compared to developed countries.⁴

MATERIAL AND METHODS

A total of 184 donor liver biopsies were performed during the one year from July 2011 to July 2012. These were performed pre-transplant as the part of the pre-transplant work-up. The biopsies were sent to the histopathology department in 10% neutral buffered formalin, fixed for at least 6 hours and submitted for routine histopathology processing overnight. Slides made from the paraffin blocks were stained for Haematoxylin & Eosin. As per our protocol, a set of liver special stains were also performed, namely, periodic acid Schiff (PAS), PAS with diastase, Perl's stain, Masson trichrome, reticulin and orcein.

The biopsies were studied for all the parameters defined by our liver biopsy reporting protocol; however only 3 relevant parameters are analysed for this study, i.e. steatosis, inflammation (portal and lobular) and fibrosis stage. Steatosis was categorized as: less than

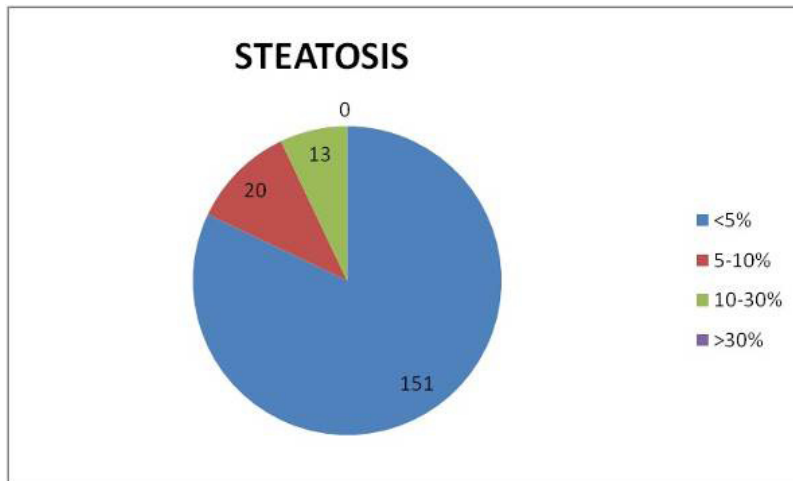


FIG. 1: Distribution of steatosis categories in donor liver biopsies (n=184).

5%, 5-10%, >10-30% and >30%. Inflammation was categorized as minimal, mild, moderate and severe, while the inflammatory infiltrate was categorized as lymphomononuclear (if consisting of lymphocytes and plasma cells) and mixed (if consisting of a mixture of lymphomononuclear cells, eosinophils and neutrophils). Masson trichrome stain was used for assessment of fibrosis. Fibrosis was assessed on a scale of 1 to 6. The fibrosis stage was defined according to the modified Ishak's system: Stage 0 = no fibrosis; Stage 1 = expansion of some portal areas with or without septa, Stage 2 = expansion of most portal areas with or without septa, Stage 3 = expansion of most portal areas with occasional portal to portal bridges, Stage 4 = expansion of portal areas with marked bridging, Stage 5 = marked bridging with occasional nodules and Stage 6 = cirrhosis, (probable or definitive).⁵

RESULTS

Steatosis

Of the 184 biopsies, 151 (82%) showed less than 5% steatosis (Figure 1). The remaining 33 (18%) biopsies showed steatosis of more than 5%, with 20 (10%) showing 5-10% steatosis while 13 (7%) showed >10-30% steatosis. None of the biopsies showed steatosis more than 30%. The various categories of steatosis are illustrated in Figure 2.

Inflammation

A substantial number (74/184; 40.2%) of biopsies showed some degree (minimal to mild) of portal inflammation. 25.5% (47/184) of all biopsies showed minimal portal inflammation, while 14.6% (27/184) showed mild portal inflammation. The predominant pattern of inflammation was portal: 91.8% (68/74) showed only portal

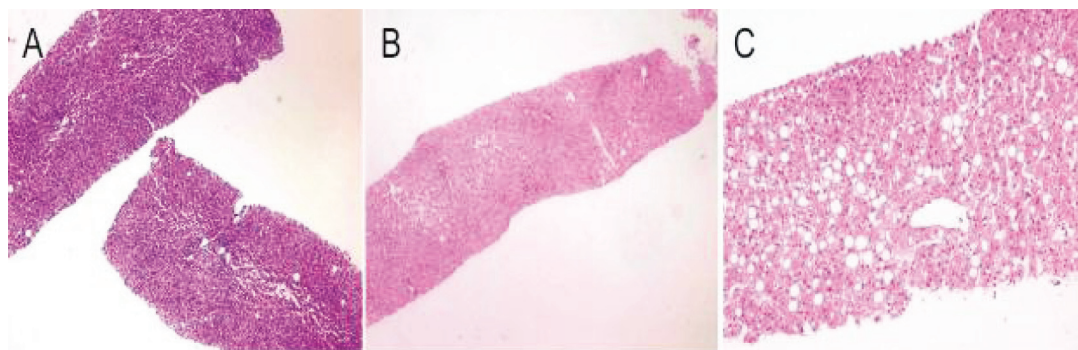


FIG. 2: Liver biopsies showing steatosis categories. (A) <5%, (H&E, 100x), (B) 5-10%, (H&E, 100x), (C) 20% (H&E, 400x).

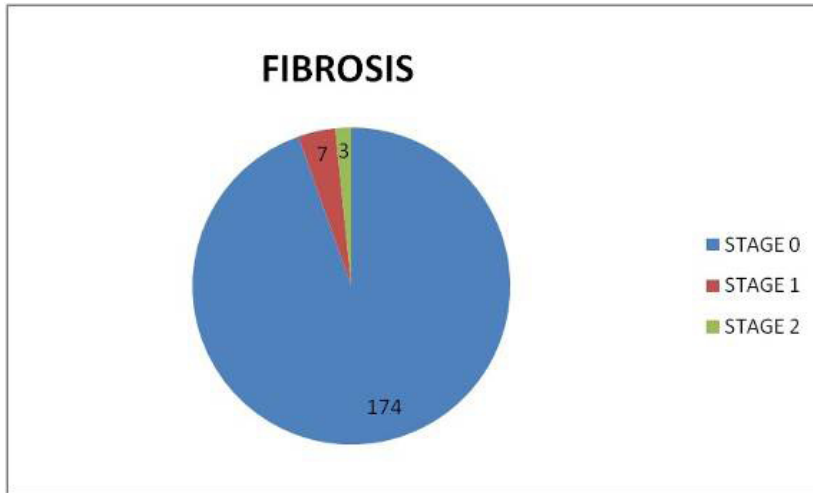


FIG. 3: Prevalence of fibrosis stages encountered in donor liver biopsies (n=184)

inflammation. Five showed inflammation in both portal area and lobules, while an isolated case had only lobular inflammation. The inflammatory cells were lymphomononuclear in all but one case, which had a few neutrophils and rare eosinophils in the infiltrate.

Fibrosis

An overwhelming majority (94.5%, 174/184) of the biopsies showed no fibrosis (stage 0). Of the 10 that showed fibrosis, 7 (3.8%) showed stage 1 fibrosis while 3 (1.6%) showed stage 2 fibrosis (Figure 3). None of the biopsies showed fibrosis of stage 3 or more. The findings are illustrated in Figure 4.

DISCUSSION

Donor liver biopsy findings, together with other investigative findings, assist transplant physicians and surgeons in deciding on the suitability of the donor. Similar to the study by Savas *et al*, our

institution performs donor liver biopsy only in selected cases, which constitute around 20-30% of all donors. A study by Brown revealed that most centres in USA also perform liver biopsy in selected cases.⁶ The primary purpose of the liver biopsy evaluation is to reduce donor mortality and provide a suitable graft for the recipient. The presence of steatosis, inflammation or fibrosis are associated with poor graft survival.⁷ Also, one biopsy is considered sufficient and representative of the entire liver.

The problem of metabolic syndrome is continuously increasing across the world and leading to an increased incidence of non-alcoholic fatty liver disease (NAFLD). The existing prevalence of NAFLD in the general Indian population is estimated to be between 9% to 32%. This population may have subtle changes which may be missed by radiological modalities but can be easily detected by a liver biopsy.⁸

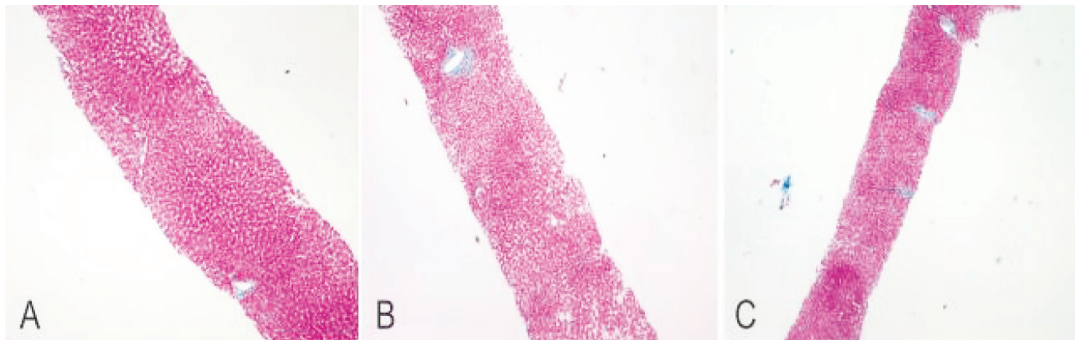


FIG. 4: Donor liver biopsies showing fibrosis. Masson Trichrome stain. Fibrosis is demonstrated by green colour. (x100) (A) Stage 0, (B) Stage 1, (C) Stage 2.

Steatosis assessment is important because a steatotic liver has poor microcirculation, resulting in ischemic damage and releases TNF- α , which damages the lungs.⁹ We found steatosis involving >5% of the parenchyma in 18% of the donors, which was less than that seen by Cuomo *et al* (30%), Minervini *et al* (37%) and Tran *et al* (38%).¹⁰⁻¹² This prevalence was similar to that seen by Savas *et al* and Nadalin *et al*.^{1,6} The cut-off value of steatosis for acceptability as a donor is usually 30%, but some centres have the cut-off set at 20%. Heller *et al* tried to determine the usefulness of steatosis assessment by frozen section examination, but found that around 34% showed discrepancies between frozen and permanent sections.¹³ Such a difference could be clinically detrimental, hence in our centre we discourage the hepatologist from using frozen section for donor evaluation. However we often use a short programme (6 hours) for processing, which helps to get the slides ready faster for examination by the transplant pathologist.

Radiological steatosis assessment is considered to be less sensitive at lower values of steatosis; however it correlates well when the steatosis is more than 30%.¹ Ultrasonographic steatosis assessment has low negative predictive value and low sensitivity.¹⁴ The lack of any case in our study with steatosis more than 30% could be because most of these patients were identified on radiology and were not evaluated further. In donors with NAFLD, diet modification also leads to reduction in steatosis, and the patient may become eligible donors in future.⁶

We detected fibrosis in 5.4% of the donor biopsies, which was significantly less than that seen by Coumo *et al*.¹⁰ However, this was more than that seen by Savas *et al* (3%) and Nadalin *et al* (<1%).^{1,6} This reduced prevalence of fibrosis in recent studies could be due to prior fibrosis assessment by radiology (e.g. Fibroscan) in the protocol for assessing the fitness of donors. Any amount of fibrosis would result in the donor being deemed unsuitable for transplant.

A significant number of donors showed some degree of inflammation (40%). This was more than what was encountered by Cuomo *et al* (25%).¹⁰ However, most of the donors had only minimal portal inflammation, which was a non-specific finding and did not result in deferral of the donor. Minimal non-specific inflammation can be seen in the general population without any other biochemical or clinical finding. Subtle fibrosis which may be missed on fibroscan can be caught on liver biopsy using the various

connective tissue stains, like Masson trichrome used by us. Although not included under the purview of the study, no granuloma was seen in any of our donors, as had been reported by Tran *et al*.¹² A study by Markin *et al*¹⁵ found intra-operative liver biopsies to be of limited use, but did find a correlation between elevated PT and AST and hepatocyte necrosis. The high prevalence of tuberculosis which has epidemic proportions in India also makes it important not to miss the presence of tubercular granulomatous lesions.¹⁶ These lesions can only be detected by using a liver biopsy and can easily be missed on radiology. Although no granulomatous lesion was detected in any of our donors, the high prevalence of the disease in the population cannot be overlooked.

Most of the donors having any abnormality on the donor biopsies were not accepted for transplant. Donors having even mild inflammation or even stage 1 fibrosis were not accepted. It is therefore not possible to determine the impact that these changes could have had on the outcome of the transplantation. Although there is a definite shortage of cadaveric donors in India, keeping in mind the health of both donor and patient, it is still significant to have the best possible donor, with no significant abnormality in the donated organ. Thorough and strict screening criteria are important to achieve this objective. The Human Organ Transplant Act in India also restricts donation from related donors in order to prevent any sort of organ trade.

Liver biopsy, being an invasive procedure, is inherently associated with complications. However, these complications occur in less than 1% of the biopsied and should not deter from undertaking the procedure, as the information obtained is vital for two lives (donor and recipient).⁶ The same views have been expressed in the review article by Nadalin *et al*.¹⁷

It has been discussed above, although radiological advances have reduced the requirement of liver biopsy for steatosis and fibrosis assessment, there is still a lot of information that can be provided by the liver biopsy, and at a relatively cheap cost. Also, considering the morbidity and mortality associated with liver transplantation, it is imperative that all the decisions are taken with utmost care, for which liver biopsy is a cornerstone. Our experience would suggest that donor liver biopsy is still of value, especially in a developing country.

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