

ORIGINAL ARTICLE

Steatosis, inflammation, fibroprogression, and cirrhosis in remnant liver post-liver donation

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Introduction

There is a definite mismatch between cadaveric donation rates and those who need an organ transplant world over.^{1,2} Lack of robust deceased donor programs in Asia paved the way for living donor liver transplantation which has now become standard of care in the East as well as the West. With around three decades of experience, although the short-term outcomes and Health Related Quality Of Life (HRQOL) of living donors have been described in literature, the long-term health consequences, especially those pertaining to remnant liver, are yet to be fully understood. Liver donors do not have a universally accepted follow-up protocol, even though guidelines recommend lifelong follow-up with annual checkup.^{2,3} Many of them, after donation, do not

Abstract

Background and Aim: This is a cross-sectional observational study conducted on living liver donors focusing on “long-term remnant liver health” specifically looking at steatosis, inflammation, and fibrosis using multiparametric ultra sonological evaluation and noninvasive blood tests.

Methods: Multiparametric ultrasound evaluation included assessment of shear wave elastography (fibrosis), sound speed plane wave ultrasound, attenuation plane wave ultrasound (steatosis), and viscosity plane wave ultrasound (inflammation). Blood test based APRI and FIB-4 were calculated. Liver biopsy was performed if noninvasive evaluation pointed toward clinically relevant fibro progression (F4).

Results: Out of 36 donors, significant fibrosis (>F2) was found in 11 donors (30.5%), seven donors (19.4%) had severe fibrosis (>F3), and two donors had shear wave elastography values suggestive of cirrhosis(F4). Of these two, one donor was extensively evaluated and was found to have biopsy proven cirrhosis with endoscopic evidence of portal hypertension. The prevalence of fatty liver disease in our study group was 50%.

Conclusion: We report the first liver donor cohort with fibroprogression and cirrhosis occurring in the remnant liver. Our donor cohort with a significant proportion having steatosis and fibroprogression underscores the importance of regular follow-up of liver donors and evaluation of remnant liver.

undergo any sort of evaluation of remnant liver health. The prevalence of metabolic dysfunction-associated fatty liver disease (MAFLD) in general population is around 25–30% globally and as high as 49.8% in some regions of India.^{4–6} Although all liver disease etiologies are ruled out at the time of liver donation, living liver donors are prone to MAFLD which can evolve from bland steatosis to inflammation and fibrosis. Obese donors are optimized by motivating them to lose weight to facilitate donation, but long-term outcome of such donors is unknown. Many transplant units in the past relied on imaging (CT/MRI) for steatosis assessment of donors without embarking on pre-donation biopsy; this protocol may miss biochemically silent yet histopathologically active MASH. Regular donor follow-up does not happen in most liver units and data on liver health among

donors, with focus on steatosis, inflammation, and fibrosis, are scarce in the published literature.

Methods

This is a cross-sectional observational study aiming at collecting data pertaining to remnant liver health of living liver donors who attended a liver clinic between October 2021 and June 2022. Institutional review board approval was obtained; informed consent was obtained from the participants. Living liver donors who were between 18 and 60 years of age were included. Patients with history of chronic alcohol intake, biliary complications after liver donation, or recent COVID infection were excluded. A total of 36 donors were included in the study. Anthropometric evaluation—height, weight, body mass index (BMI), and waist circumference were obtained and categorized based on WHO criteria as applicable to South Asian population, namely, overweight—BMI between 23 and 24.9 kg/m², obesity—BMI greater than 25 kg/m², and waist circumference cutoff of ≥ 102 cm for men and ≥ 88 cm for women.

Ultrasonographic evaluation was done using multi-parametric ultrasound machine (Super Sonic Imagine, Aix en Provence, France). The liver stiffness, steatosis, and viscosity were assessed by a senior radiologist and relevant blood investigations including liver function test (LFT) and platelet count were done based on which APRI and FIB4 scores were calculated. 2D-shear wave elastography measurement using Super Sonic Imagine is an efficient and accurate modality to assess liver fibrosis.^{7–10} The shear wave elastography (2D-SWE), sound speed plane ultrasound (SSp.PLUS), attenuation plane wave ultrasound (Att.PLUS), and viscosity plane wave ultrasound (Vi.PLUS) were assessed using the Aixplorer MACH 30 system, as per standard recommendations.^{7,11}

Data were analyzed using MedCalc v18.2.1 (MedCalc Statistical Software version 18.2.1). Independent t-test or Mann–Whitney test was used to check for significance between groups; *P* value < 0.05 was considered to be statistically significant.

Results

Demographic data of living liver donor population. The study included data from 36 living liver donors with a mean age of 41.5 years; 14 were males (38.9%) and 22 were females (61.1%). Twenty-eight donors (77.4%) had BMI more than or equal to 23 kg/m² and 24 (66.6%) were obese (BMI ≥ 25 kg/m²). Only four out of 14 males had waist circumference more than 102 cm, whereas majority of females (21 out of 22) had waist circumference more than 88 cm.⁸ Twenty-seven (75%) individuals donated their liver within the past 5 years and nine (25%) individuals donated before 5 years. The categorization of donors into more than 5 years and less than 5 years post-donation was done since MAFLD and histological progression occurs over time. We included donors from the year 2010 onward; at the time of donation, donors were chosen only if they did not have significant steatosis (less than 10% as assessed by CT/MRI). The fatty liver which evolves/progresses after donation is likely to have more pronounced changes in the more than 5 years category. Twenty-one donors did their liver donation on an elective basis while 15 donors

Table 1 Demographic data of donors

Sl. no	Parameter	Group	N (%)
1	Age	≤ 25	7 (19.45)
		26–50	21 (58.33)
		> 50	8 (22.22)
2	Sex	Male	14 (38.89)
		Female	22 (61.11)
3	BMI	18.5–22.9	8 (22.22)
		23.0–24.9	4 (11.11)
		> 25	24 (66.67)
4	Waist circumference	Male < 102 cm	10 (27.78)
		≥ 102 cm	4 (11.11)
		Female < 88 cm	1 (2.78)
		≥ 88 cm	21 (58.33)
5	Years since donation	5 years and below	27 (75)
		> 5 years	9 (25)
6	Timing of surgery	Elective	21 (58.33)
		Emergency	15 (41.67)
7	Type of graft donated	Right lobe	33 (91.67)
		Left lobe	3 (8.33)

had an emergency indication for donation. Majority of them (91.7%) had a right lobe graft donation, whereas very few (8.3%) had donated a left lobe graft (Table 1). Regarding comorbidities in donors, at the time of donation, donors were rejected if they had any comorbidity. As per donor selection protocol, healthy adults without comorbidities alone were chosen as donors. In our cohort of 36 donors, three currently have dyslipidaemia and two developed systemic hypertension—all five of them belonging to the more than 5 years post-donation category. Donor surgery and transplant were done in other centers from the year 2010 onward and hence we do not have access to the weight/BMI at the time of donation. Current BMI is shown in Table 1.

Predonation biopsy was not routinely included in the donor protocol a decade ago and even now biopsy is done only in cases where imaging reveals borderline findings on steatosis. Plain CT study looking at attenuation differences between Liver and Spleen (L–S and L/S) is the most widely used technique to assess donor steatosis. MRI-based PDFF (Proton Density Fat Fraction) is in vogue currently. Steatosis assessment based on imaging alone may not be accurate always. We do not have Time Zero biopsy data, since the surgery was done in other centers and we do not have access to the same. Hence, biochemically silent (normal transaminases in LFT) yet histopathologically active disease would have got missed out when donor steatosis assessment was based on imaging.

Noninvasive serum markers and sonological parameters in donor population. Liver donors were stratified into two groups based on the timing of donation as within 5 years post-donation and more than 5 years post-donation (Table 2). The mean platelet count of donor cohort was 2.55 ± 0.56 lakhs/mm³. The mean platelet count of within 5 years post-donation and more than 5 years post-donation were 2.67 ± 0.48 lakhs/mm³ and 2.21 ± 0.67 lakhs/mm³, respectively,

Table 2 Noninvasive serum markers and sonological parameters in donors

Sl. no.	Parameter	Group	Mean (SD)	Range	P value
1	APRI	Up to 5 years	0.2333 (0.1074)	0.10–0.50	<i>P</i> = 0.1721
		>5 Years	0.4000 (0.4183)	0.20–1.50	
		Total	0.2750 (0.2322)	0.10–1.50	
2	FIB-4	Up to 5 years	0.7304 (0.3206)	0.30–1.41	<i>P</i> = 0.0031
		>5 Years	1.5533 (1.5453)	0.78–5.66	
		Total	0.9361 (0.8676)	0.30–5.66	
3	2 D- SWE (kPa)	Up to 5 years	7.0852 (2.5787)	5.0–16.2	<i>P</i> = 0.4870
		>5 Years	8.0000 (3.0245)	4.7–14.1	
		Total	7.3139 (2.6818)	4.7–16.2	
4	Viscosity PLUS (Pa.s)	Up to 5 years	2.3000 (0.5371)	1.70–3.60	<i>P</i> = 1.00
		>5 Years	2.3000 (0.5590)	1.60–3.20	
		Total	2.3000 (0.5345)	1.60–3.60	
5	Sound speed PLUS (m/s)	Up to 5 years	1536.7407 (32.4838)	1495–1624	<i>P</i> = 0.7011
		>5 Years	1530.7778 (19.0839)	1509–1567	
		Total	1535.2500 (29.5629)	1495–1624	
6	Attenuation PLUS (dB/cm/MHz)	Up to 5 years	0.4907 (0.1152)	0.27–0.67	<i>P</i> = 0.5811
		>5 Years	0.5167 (0.1379)	0.26–0.66	
		Total	0.4972 (0.1197)	0.26–0.67	

and the difference was found to be statistically significant. APRI (AST to Platelet Ratio Index) and FIB-4 (Fibrosis-4) scores were calculated for all the donors, and assessed for both the groups separately. The mean APRI score for the total donor population was 0.2750 ± 0.23 with a range of 0.10–1.50. The mean APRI scores for within 5 years and more than 5 years post-donation groups were 0.2333 ± 0.10 and 0.4000 ± 0.41 , respectively (*P* value 0.17). The mean FIB-4 score for the total donor population was 0.9361 ± 0.86 with a range of 0.30–5.66. The mean FIB-4 scores for within 5 years and more than 5 years post-donation groups were 0.7304 ± 0.32 and 1.5533 ± 1.54 , respectively (*P* value-0.003). Although APRI scores did not show any significant difference in fibrosis between two groups, FIB4 values were suggestive of more fibrosis occurring in more than 5 years post-donation group and this difference was found to be statistically significant. The mean 2D-SWE value for the total donor population was 7.31 ± 2.68 kPa with values ranging from 4.7 to 16.2 kPa. The mean 2D-SWE values for within 5 years and more than 5 years post-donation groups were 7.08 ± 2.57 kPa and 8.0 ± 3.02 kPa, respectively (*P* value 0.487). The mean Viscosity PLUS value for the total donor population was 2.30 ± 0.53 Pa.s with values ranging from 1.6 to 3.6 Pa.s. The mean Viscosity PLUS values for within 5 years and more than 5 years post-donation groups were 2.30 ± 0.53 Pa.s and 2.30 ± 0.55 Pa.s, respectively (*P* value 1.0). The mean Sound Speed PLUS value for the total donor population was 1535.25 ± 29.56 m/s within a range of 1495–1624 m/s. The mean SSp PLUS values for within 5 years and more than 5 years post-donation groups were 1536.74 ± 32.48 m/s and 1530.77 ± 19.08 m/s, respectively (*P* value 0.701). The mean Att.PLUS value for the total donor population was 0.4972 ± 0.1152 dB/cm/MHz with a range of 0.26–0.67 dB/cm/MHz. The mean Attenuation PLUS values for within 5 years and more than 5 years post-donation groups were 0.4907 ± 0.1152 dB/cm/MHz and 0.5167 ± 0.13 dB/cm/MHz, respectively (*P* value—0.5811).

Table 3 Staging using sonological parameters

Sl. no.	Parameter	Group	N (%)
1	2D SWE	F0-F1	25 (69.44)
		F2	4 (11.11)
		F3	5 (13.89)
		F4	2 (5.56)
2	Attenuation PLUS	Significant steatosis	18 (50.00)
		No significant steatosis	18 (50.00)
3	Sound speed PLUS	Significant steatosis	14 (38.89)
		No significant steatosis	22 (61.11)

Staging using sonological parameters. Donors were grouped into different fibrosis stages according to 2D-SWE values (Table 3). The cutoff for various fibrosis stages were 7.1 kPa (F2), 9.2 kPa (F3), and 13.0 kPa (F4).^{7,9} Significant fibrosis (F2 or more) was seen in 11 donors (30.5%), of which two donors (5.5%) had 2D-SWE values in cirrhotic range (>13 kPa). Of the two donors with SWE value more than 13 kPa, one underwent upper GI endoscopy, liver biopsy, and computerized tomographic (CT) scan of liver, whereas the other donors did not give consent for liver biopsy or endoscopy. Cirrhosis was histopathologically confirmed in a 56-year-old donor who had endoscopic evidence of portal hypertension. Eighteen donors (50%) had significant steatosis as per Attenuation PLUS and 14 donors (38.8%) had significant steatosis as per Sound speed PLUS.

Discussion

The medical, psychosocial, and quality of life parameters in living liver donors have been assessed over short- and long-term period in various studies worldwide. Ladner et al. assessed the long-term quality of life after liver donation in the Adult to Adult Living Donor Liver transplantation cohort study (A2ALL) and

found that most of the living donors maintained above average HRQOL 11 years post-donation.¹⁰ Dew et al. reviewed the literature regarding long-term medical and psychosocial outcomes in living liver donors and defined “long term” as beyond first year post-donation and noted that although long-term mortality and morbidity appear to be low, there is dearth of data on long-term remnant liver health.¹²

As per the International Liver Transplant Society (ILTS) guidelines, all donors should have regular clinical monitoring and follow-up for at least 2 years post-donation and preferably for lifetime with annual healthcare checkup.² Unfortunately, these guidelines are not strictly adhered to in clinical practice. Prospective studies confirmed safety of living liver donation and long-term follow-up may contribute to donor outcomes.¹³ In a multicentric study on Asian living liver donors by Chung et al., long-term follow-up beyond 3 months was available in only 15% of donors, and they suggested long-term follow-up of donors.¹⁴ Robert S Brown Jr. et al. in their study analyzed follow-up data of donors in A2ALL study, and found that donor follow-up was excellent in the first year post-donation, but decreased with time.¹⁵

There is a predominance of middle-aged individuals in our study cohort, with a male to female ratio of approximately 1:2. Significant proportion of donors were overweight as per Asian criteria cutoff for BMI value, with around two-third of donors being obese (BMI > 25 kg/m²). Also majority of the females had a waist circumference above the stipulated cutoff of having metabolic syndrome. Significant prevalence of obesity and metabolic syndrome in donor population puts them under risk for development and progression of non alcoholic fatty liver disease (NAFLD).¹⁶ This highlights the importance of regular follow-up along with strict measures to maintain ideal body weight by diet and lifestyle measures.

In our study cohort, the mean platelet count was found to be reduced in more than 5 years post-donation group in comparison to up to 5 years post-donation group. In a long-term follow-up of living liver donors by Murad et al., the only laboratory abnormality noticed was a significant decrease in platelet count.¹⁷ Although the mean APRI value in donors more than 5 years post-donation was more than that of donors up to 5 years post-donation, the difference was not statistically significant. Interestingly the FIB-4 value showed a statistically significant difference between these groups suggesting possibility of significant fibrosis in more than 5 years post-donation group.¹⁸

The mean 2D-SWE value was found to be 7.3 kPa, which is suggestive of significant fibrosis (>F2).⁹ Although the mean 2D-SWE value in more than 5 years post-donation group (8.0 kPa) was more than that of within 5 years post-donation group (7.0 kPa), the difference was not statistically significant. Viscosity PLUS provides information regarding the shear wave dispersion within a tissue, allowing viscosity measurements in a selected region of interest and thereby helps to roughly estimate the inflammation going on in the liver.¹⁹ A value more than 1.8 Pa.s is considered to be suggestive of significant inflammatory activity in liver. The mean Vi PLUS value in the study group was 2.3 Pa.s with no significant difference between the two groups. This is suggestive of a possible ongoing steatohepatitis in these donors. Sound speed PLUS (SSp PLUS) and Attenuation PLUS (Att PLUS) helps to noninvasively assess

the degree of hepatic steatosis. A recent study by Alexandru Popa et al. suggested a cutoff value for predicting significant steatosis using Att PLUS and SSp PLUS as 0.5 dB/cm/MHz and < 1524 m/s, respectively.^{11,20} As per this reference, 50% of donors have significant steatosis according to Att PLUS cutoff, and 38.8% of donors have significant steatosis according to SSp PLUS cutoff value. In other words, 40–50% of donors in our study group were found to have significant steatosis, which tallies with the prevalence of fatty liver disease in general population in this part of the world.¹¹

In a meta-analysis by Herrmann et al., 2D-SWE has good to excellent performance for the noninvasive staging of liver fibrosis; the proposed cutoff for diagnosing significant fibrosis (≥F2) is 7.1 kPa, regardless of disease etiology.⁹ And for diagnosing severe fibrosis (F3) and cirrhosis (F4), the cutoff values are 9.2 and 13.0 kPa, respectively. In our study population, 11 donors (30.5%) were found to have significant fibrosis.

Two out of 36 donors in our study had 2D-SWE values above 13 kPa suggestive of cirrhotic transformation. The 56-year-old lady who had donated right lobe in 2009, underwent complete evaluation. CT scan revealed surface irregularity and nodularity (Fig. 1), upper GI endoscopy revealed Grade II varices, and liver biopsy confirmed cirrhosis (Fig. 2a,b). Serovirology workup for delineating etiology turned out to be negative. Etiological workup done in 2009 prior to donation also was

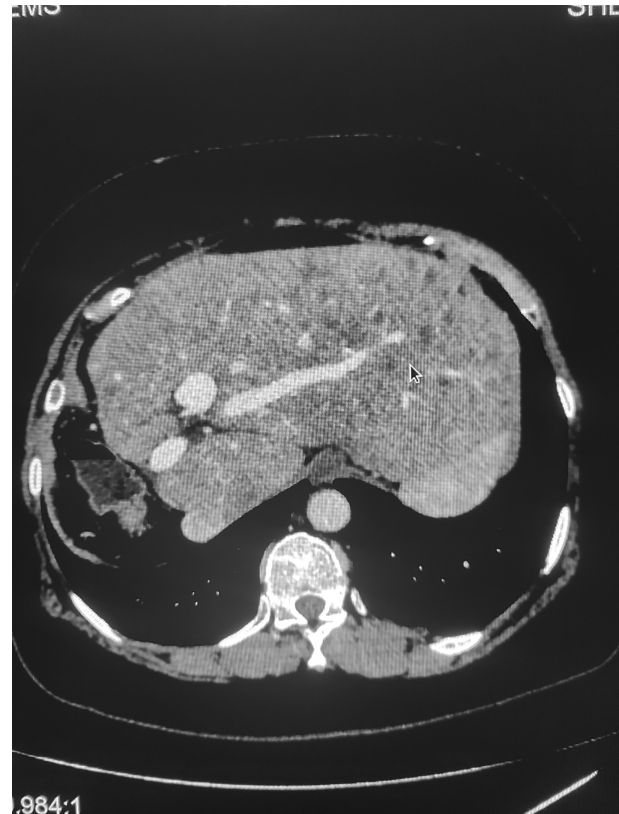


Figure 1 Axial computerized tomographic section of upper abdomen in venous phase. The remnant liver shows altered attenuation with surface appearing irregular with multiple regenerative nodules.

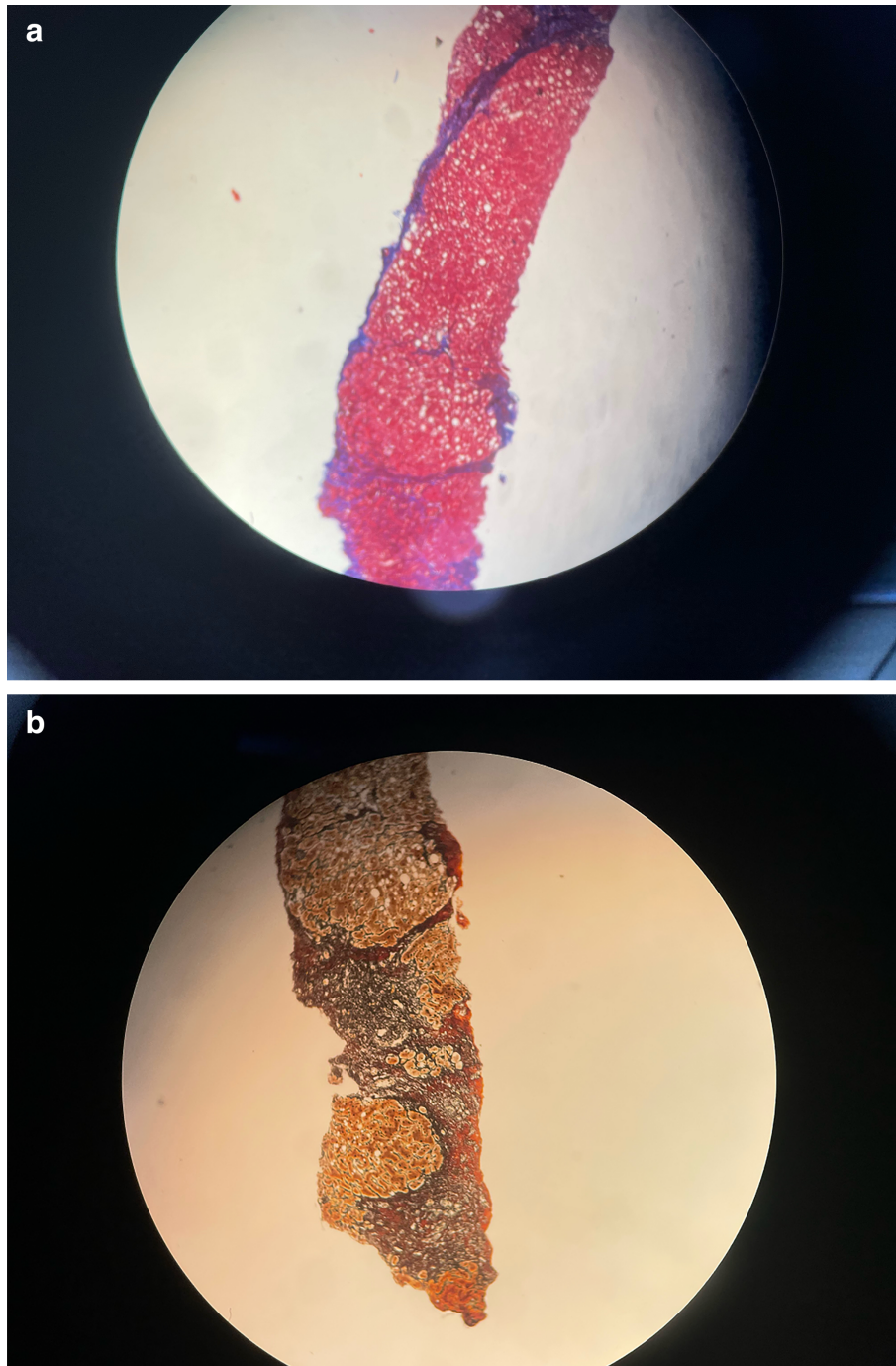


Figure 2 (a) Liver biopsy: Periodic Acid Schiff (PAS) stained section (50 \times)—Hepatocytes showing PAS positivity arranged in the form of nodules with widened portal tracts. (b) Masson's Trichrome (MT) stained section (100 \times) highlighting architectural distortion with hepatocytes arranged in nodular form and fibrotic bands.

negative. She had gained 16 kg in 12 years, with current BMI of 32.4 kg/m²; these along with liver biopsy findings, etiology was confirmed to be NASH. She was initiated on Carvedilol and put in HCC surveillance pathway. Since the duration post-donation is only 12 years and given the fact that natural history of NAFLD from bland steatosis to fibro progression and cirrhosis generally

takes two to three decades, our case raises the suspicion that fibroprogression due to NAFLD can occur at faster pace in donors.²¹ The second donor, with 2D SWE value of 16.2 kPa, 45-year-old lady who donated her right lobe 9 years back, did not give consent for liver biopsy. Regarding recipients of the donors who developed cirrhosis, one is 14 years posttransplant

enjoying good quality of life and does not have clinically relevant fibroprogression in his grafted liver. The second recipient developed hepatic venous outflow obstruction, immediate post-transplant period which was initially managed with stenting of the outflow tract; he died third year posttransplant.

The short-term outcomes and HRQOL of living liver donors are well known. Although all parenchymal and cholestatic etiologies are ruled out while selecting a donor, lifestyle liver disorders, especially NAFLD and AFLD can cause histopathological progression in the remnant liver.

Conclusion

The short-term outcomes and HRQOL of living liver donation are well known. Although all parenchymal and cholestatic etiologies are ruled out while selecting a donor, lifestyle liver disorders, especially MAFLD can cause histopathological progression in the remnant liver. Follow-up protocols for liver donors are highly variable; many liver units do not offer a lifelong follow-up for donors. Our donor cohort with a significant proportion developing fibrosis, that too in a short span of a decade after donation underscores the importance of establishing stringent guidelines for long-term donor follow-up.

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