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Detecting Hepatic Steatosis Among Potential Liver Donors by Using Non-Invasive Methods

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Author`s Contribution

[1,3]Substantial contributions to the conception or design of the work; or the acquisition, [2,4,6]Drafting the work or revising it critically for important intellectual content Final approval of the study to be published ,[5,7]Active participation in active methodology, Statistical analysis.

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ABSTRACT

Objective: To evaluate the effectiveness of non-invasive methods in detecting hepatic steatosis among potential liver donors.

Methodology: This cross-sectional descriptive study included 67 potential liver donors, recruited via consecutive non-probability sampling over a two-year period (2021-2023). Data collection involved a semi-structured questionnaire covering demographics and biochemical indicators like serum bilirubin, cholesterol levels, ALT, AST, platelets, INR, and GGT. Hepatic steatosis was assessed using Fasting Lipid Profile, Fibroscan/Shearwave ultrasonography, and Liver Attenuation Index (LAI) from CT scans. Descriptive statistics were applied, and gender-based variations in biochemical markers, CAP, and LSM were analyzed using independent t-tests. Chi-square tests evaluated gender differences in steatosis identified by pathology and CAP. A significance level of $P < 0.05$ was used.

Results: Of the 67 donors, 49 were males, with a mean age of 30.1 ± 8.8 years. Pathology revealed mild steatosis in 35 and moderate steatosis in 5 donors. Ultrasound showed normal liver echotexture in 47 subjects, while 18 had fatty liver. CAP findings indicated mild steatosis in both genders, with females showing higher values ($P = 0.02$) and more advanced steatosis ($P < 0.01$). Mean LSM was 4.6 ± 1.53 kPa, indicating normal liver stiffness. LAI findings suggested 37 donors required further evaluation. Among overweight donors, 22% had advanced steatosis compared to 14% in the healthy-weight group.

Conclusion: Ultrasound-directed CAP, LSM, and BMI are effective non-invasive tools for diagnosing hepatic steatosis in potential liver donors.

Keywords: Hepatic steatosis, liver donors, non-invasive methods, BMI, Controlled Attenuation Parameter (CAP), Liver Stiffness Measurement (LSM).

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Introduction

Hepatic steatosis is one of the frequently identified variances in liver histopathology that has been attributed to numerous environmental and genetic factors.[1] Around 1.9 million deaths annually are associated with chronic liver diseases that substantially deteriorate the quality of life.[2] Non-Alcoholic Fatty Liver Disease (NAFLD) has been reported among approximately 25% of the worldwide population with escalating prevalence over the past decade[3] that is linked with growing rate of metabolic syndrome[4]. It is perceived nowadays as the prime cause of cirrhosis that entails the need for liver transplantation.[5]

The burden of hepatic failure has enormously been increased that results in about 5000 liver transplants per annum[6]. Despite being the only treatment for end-stage liver disease, liver transplant in Pakistan is still confronted with many challenges[7]. Liver donors are likely to encounter some serious health problems like bile leakage and intestinal blockage[8]. Hepatic steatosis is considerably related to obesity, alcoholism and type-II diabetes.[9] It is an independent risk factor for poor prognosis among liver transplant recipients due to multiple resultant dysfunctions.[10]

Hepatic steatosis among non-alcoholic individuals is clinically manifested with abnormal fat accumulation in

more than 5% hepatocytes. Studies have confirmed this steatosis among more than 60% obese and 90% morbidly obese subjects.[11] The key manifestation of obesity in liver is hepatic steatosis that is demonstrated pathology by raised liver enzymes.[12] NAFLD is associated with minimal hepatic inflammation but if untreated, may progress to Non-Alcoholic Steato-Hepatitis (NASH) that is characterized with liver inflammation and fibrosis.[13] It is hence imperative to work for diagnosing hepatic steatosis among donors as it may lead to compromised outcomes among both donors and recipients. Liver biopsy is considered a gold standard for ascertaining hepatic steatosis but being invasive and costly it is not comfortably opted by majority.[14] Although interventions have been done worldwide about usefulness of non-invasive modalities but its application in Pakistan is still debatable.

The present study is therefore intended to discover the effectiveness of non-invasive methods in detecting hepatic steatosis among potential liver donors. This research would not only provide an insight into the significance of non-invasive methods to our clinicians but will also enlighten our concerned doctors to work for this inflammatory ailment well before planning liver transplantation.

Methodology

A cross-sectional descriptive study was done among 67 potential liver donors who were enrolled in the study by consecutive non-probability sampling. Data was collected over a span of 2 years (2021-2023) after the approval of synopsis from Institutional Ethical Review Board. Informed consent was taken from study participants (potential donors) for procuring the required information and subjecting them to non-invasive methods. The sample size was computed by WHO sample size calculator taking 95% confidence level, anticipated population proportion of 9.25% and 7% absolute precision. All adult potential liver donors who were not suffering from any metabolic disorder or chronic disease were included in the study. The data was gathered by means of a semi-structured questionnaire pertaining to demographics and some relevant biochemical indicators of the donors which were serum bilirubin and cholesterol levels, Alanine Amino Transferase (ALT), Aspartate Amino Transferase (AST), platelets, Blood INR and Gamma Glutamyl Transferase (GGT) tests.

All potential donors were subjected to evaluation of BMI, Liver Function Tests (LFTs) and Ultrasonography. The steatosis was further evaluated by Fasting Lipid Profile, Fibroscan/Shearwave ultrasonography and Liver Attenuation Index (LAI) calculated by Computed Tomography (CT).

Data was entered and analyzed by using SPSS version 25.0 and Microsoft Excel 2016. Descriptive statistics were applied. For age mean ± SD was calculated. Gender based differences in mean values of all biochemical indicators, CAP and LSM were determined by independent sample t-test. Gender wise variations in hepatic steatosis identified on pathology and those based on CAP were determined by applying chi-square test. $P \leq 0.05$ was considered significant.

Results

Of the 67 potential liver donors enrolled in current study, 18 and 49 were females and males respectively. The mean age of the study subjects was 30.1 ± 8.8 years. Most of them were 21-25 years old as depicted below in Figure 1.

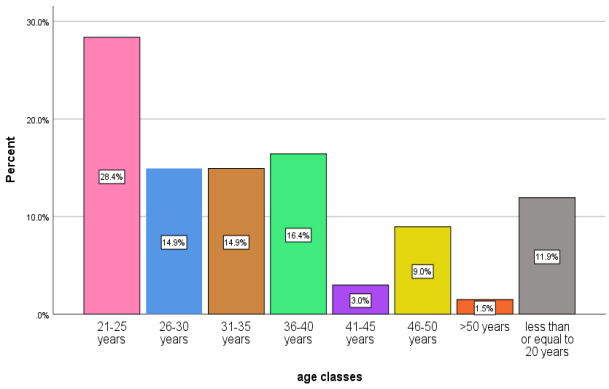

Figure 1. Age groups of potential donors.

On calculating BMI, about 77% females and 57% males were overweight as illustrated below in Table I.

Table I: BMI of study participants.

Gender	Body Mass Index (BMI)			Total
	Underweight (<23)	Healthy weight (23-24.9)	Overweight ≥ 25	
Males	10	11	28	49
Females	01	03	14	18
Total	11	14	42	67

Grades of steatosis identified among the potential donors on pathology are depicted below in Figure 2.

Gender-wise variation in steatosis as ruled out on pathology was determined on applying chi-square test as presented below in Table II.

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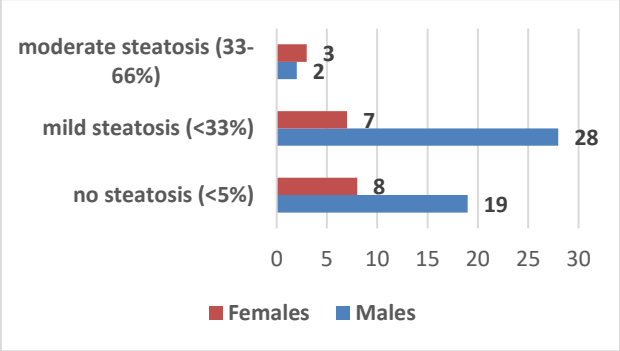

Figure 2. Grading of steatosis on Pathology

Table II: Gender-based differences in Hepatic steatosis (on pathological findings)

Gender	Hepatic steatosis		Total
	No steatosis	Mild-moderate steatosis	
Males	19	30	49
Females	8	10	18
	$X^2 = 0.17$	P > 0.20	67

Ultrasonographic findings revealed normal parenchymal echotexture of the liver among 47 subjects while mild fatty parenchymal echotexture with smooth margins were seen among 18 potential donors. Only 2 were diagnosed with moderate steatosis (grade-II).

Gender-based mean variations in biochemical indicators, Liver Stiffness Measurement (LSM) and Controlled Attenuation Parameter (CAP) of potential donors are illustrated below in Table III.

Of the 67 potential donors, 29 had acceptable LAI while 37 were determined to need further evaluation before liver donation as shown below in Figure 3.

CAP findings revealed advanced steatosis more among females as shown in Table IV. The relationship of body weight with hepatic steatosis on CAP findings revealed that greatest proportion (22%) of the donors from overweight category had advanced steatosis as reflected below in Table V.

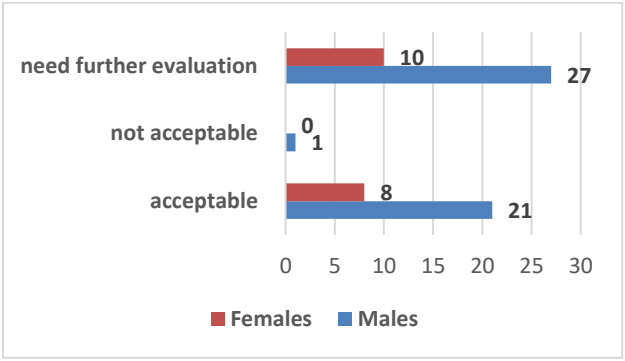

Figure 3. LAI findings.

Table IV: Gender-wise differences in CAP-based hepatic steatosis

Gender	CAP-based Hepatic steatosis		Total
	mild steatosis	advanced steatosis	
Males	44	5	49
Females	11	7	18
	$X^2 = 7.48$	P < 0.01	67

Table V: Relationship of BMI with CAP-based steatosis.

BMI	CAP-based Hepatic steatosis		Total
	mild steatosis	advanced steatosis	
<23 (underweight)	10	1 (9%)	11
23-24.9 (healthy weight)	12	2 (14%)	14
≥25 (overweight)	33	9 (22%)	41

Discussion

Of the 67 potential liver donors in the current study, 42 were found to be overweight and only 14 had normal body weight. Data collected from the National Health and Nutrition Examination Survey (NHANES) reflected the association of fatty liver and hepatic fibrosis with elevated Fat Mass Index (FMI).[15] Various metabolic disorders like type-II diabetes, hypertension, dyslipidemia and obesity

Table III: Gender-wise differences in mean values of biochemical indicators, LSM and CAP of donors.

Biochemical indicators / parameters (normal levels)	Overall (Mean ± Standard Deviation)	Gender-wise mean values (Mean ± Standard Deviation)		P-value
		Males (n = 49)	Females (n = 18)	
Serum cholesterol (< 200 mg/dl)	175.7 ± 34	176.4 ± 35.4	174.05 ± 30.8	0.890
Serum bilirubin (0.2 – 1.3 mg/dl)	0.45 ± 0.21	0.53 ± 0.21	0.36 ± 0.15	*0.002
ALT (7-55 U/ L)	40 ± 29.9	41.3 ± 17.01	36.11 ± 51.3	0.530
AST (8-48 U/L)	27.3 ± 14.2	27.5 ± 11.9	26.5 ± 19.6	0.800
Platelets (150-450 × 10^3/ µL)	196 × 10^3 ± 117.9	187.91×10^3 ± 105.6	219.6×10^3 ± 147.4	0.330
INR (0.9 -1.3)	1.0 ± 0.09	1.01 ± 0.08	1.0 ± 0.09	0.660
GGT (6-50 IU/L)	35.5 ± 20.8	35.4 ± 18.01	35.6 ± 27.7	0.970
CAP (≤267 dB/L) indicates mild hepatic steatosis	237.6 ± 40.1	230.8 ± 36.1	256.2 ± 45.3	*0.020
LSM (2-7 kPa)	4.6 ± 1.53	4.5 ± 1.0	4.71 ± 2.4	0.610

are linked with rising prevalence of 20-30% of hepatic steatosis essentially in United States.[16] A similar study carried out among extensive male population of United States revealed that of the varied patterns of obesity, expansion of waist circumference seemed to be substantially connected with occurrence of NAFLD.[17]

About 63% of the potential donors in the present study were overweight that illustrates the need for change in dietary habits and lifestyle modification. Hepatic steatosis in our study was determined pathologically that demonstrates mild steatosis and no steatosis among 35 and 27 potential donors respectively. Only 5 had moderate steatosis (Figure 2). On the other hand, ultrasonography illustrated the existence of moderate steatosis only among 2 people. Although steatosis one way or the other is attributed to obesity, mean serum cholesterol among our study subjects was below 200mg/dl (Table II). Measuring the level of other lipoproteins might also prove useful to establish linkage of dyslipidemia with hepatic steatosis in our scenario.

The liver enzymes like AST and ALT and serum bilirubin among our study participants were within normal range (Table II) that was reflective of normal liver functioning. These liver enzymes, if elevated provide us a clue of hepatic inflammation and ballooning.[18] A study by Verma S et al pointed out the diagnosis of NASH even in a group with normal ALT levels.[19] Keeping in view the probability pertaining to the progression of fatty liver to hepatocellular carcinoma, administration of some drugs in addition to diverse molecular and genetic modifications has also been done to manage the cases at initial stage.[20] However, a similar study by Jang BK signaled an inverse relationship of serum bilirubin with NAFLD.[21] There are chances of progression of mild steatosis to liver fibrosis and cirrhosis, so relying on a single biomarker to reach the diagnosis is not adequate.[22] Investigators must review multiple biochemical indicators of the suspected cases before reaching any conclusion.

Ultrasound by fibro scan was also done in the present study to assess CAP and LSM and their gender-based differences were also measured that verified significantly greater CAP among female subjects than those of males (Table II). The relationship of CAP-based steatosis with that of BMI of the potential donors (Table V) in current study is quite meaningful. Most of the overweight subjects (22%) were identified with advanced steatosis. Literature suggests considerable linkage of abdominal fat distribution with prevalence of hepatic steatosis.[23]

Contrary to this, another study revealed that Waist circumference to Hip circumference (WHR) is a more reliable marker to measure the distribution of central obesity.[24] Such controversies can aptly be dealt with by taking measurements of other body circumferences instead of relying only on BMI. Moreover, correlation between BMI and hepatic steatosis among our population should be projected by planning future studies.

On fibro scan, liver stiffness among all our study subjects was found to be normal (Table III) despite hepatic steatosis and obesity. Liver stiffness positively correlates with portal pressure and stiffness greater than 20 kPa may prelude to esophageal varices.[25] As LSM is ultrasound guided, this parameter is or paramount significance in monitoring the liver disease progression non-invasively.[22] Nowadays, ultrasound-based liver elastography has also been in fashion worldwide to determine the pattern of liver diseases with minimal pain and discomfort.[26] Such technologies should also be introduced in our set up for establishing their diagnostic significance.

Conclusion

Ultrasound driven Controlled Attenuation Parameter (CAP) and Liver Stiffness Measurement (LSM) and Body Mass Index (BMI) are useful non-invasive approaches to diagnose hepatic steatosis. Apart from BMI, other fat indices should also be calculated to determine their correlation with hepatic steatosis.

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