ORIGINAL ARTICLE

Comparative Study of Noninvasive Markers and Transient Elastography in Patient with Nonalcoholic Fatty Liver in Western Region of Nepal

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Abstract

Introduction: Nonalcoholic fatty liver diseases (NAFLD) is most common cause of cirrhosis. Risk factor for NAFLD are obesity, diabetes, hypertension or dyslipidemia. Main aim of the study is to find out the risk factor of NAFLD, determine the metabolic syndrome in NAFLD and compare the different noninvasive score with FibroTouch score.

Methods: This is prospective cross sectionalhospital based observationalstudy carried out in Pokhara Academy of Health Sciences (PoAHS) in period of (22nd June 2022 to 14th May 2024) two years. Any patients who visited outpatient department (OPD) with USG abdomen with fatty liver were included. Baseline characteristics were recorded, Complete blood count, Random blood sugar, Liver function tests, Fasting lipid profile, renal function tests, Hepatitis B surface antigen, Anti HCV antibody were send. Liver stiffness and hepatic steatosis was determine by FibroTouch. Noninvasive tests (score) were calculated and comparative study was performed.

Results: Mean age was 48.10±11.62 years, with male preponderance (52.2%), mean BMI was 32.44±4.82 kg/m2. Risk factor for NAFLD were obesity 47.92%, alcohol and obesity 23.50% and dyslipidemia and obesity 22.11%, USG abdomen showed majority of patients (62.7%) have grade II fatty liver with mean ultrasound attenuation parameter 276±49.16 db/m and mean liver stiffness 8.29±3.71kpa. Spearman correlation of FibroTouch score with noninvasive tests were statistically significant.

Conclusion: Fatty liver was more prevalent in middle aged male patients. Risk factor are being obesity, diabetes mellitus, dyslipidemia or metabolic syndrome. Similarly noninvasive tests like FIB4, NAFLD score, APRI score and BARD score are comparable with FibroTouch score.

Introduction

Nonalcoholic fatty liver diseases (NAFLD) comprehensive term which refers to a population in which ≥ 5% of hepatocytes display macrovesicular steatosis and there should not any identifiable cause of steatosis (eg, medications, starvation, monogenic disorders) in individuals who drink little or no alcohol (defined as < 20 g/d for women and > 30 g/d for men.[1] Nonalcoholic fatty liver disease (NAFLD) has emerged as a leading cause of liver-related morbidity and mortality worldwide.[2] In 2016, the World Health Organization estimated that more than 1.9 billion adults (39% of the adult population) were overweight and 650 million (13% of the adult population) were obese.[3] Currently, it is estimated that global prevalence of NAFLD is approximately 25%, with more than 80 million individuals affected in the United States alone.[4] There are similar rates in Asia, with an estimated pooled prevalence rate of 27.4% (95% confidence interval [CI], 23.3%–31.9%) observed.[4] MASLD (Metabolic dysfunction associated steotic liver disease) replaces the old term non-alcoholic fatty liver disease (NAFLD) and is embedded in the new consensus definition of steatotic liver disease (SLD).[1] Gold standard investigation for fatty liver is liver biopsy however it has several limitations.[5] Noninvasive tests were validated which includes non alcoholic fatty liver diseases score (NAFLD), Fibrosis 4 index (FIB 4 score), fibrometer.[5] These noninvasive tests have varied sensitivity and specificity.[5] Similarly vibration controlled elastography or transient elastography is useful for determining hepatic steatosis, degree of fibrosis and cirrhosis.[5] Metabolic syndrome[6] is defined as presence of three or more

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of following factors A) Waist circumference (Male > 102 cm or Female > 88 cm) B) Triglyceride level > 15 mg % C) High density lipoprotein cholesterol (Male < 40 mg/dl, Female < 50 mg/dl D) Blood Pressure > 135/85 mmHg E) Fasting blood glucose > 110 mg/dl. The main aim is to find out the risk factor of nonalcoholic fatty liver disease, determine their severity with different noninvasive tests and compare these scores with FibroTouch score.

Methods

This is a hospital based cross sectional observational study which was conductedamong 217 fatty liver patients who visited out patients department (OPD) in Pokhara Academy of Health Sciences (PoAHS) from period of two years (22nd June 2022 to 14 June 2024). Prior to the study ethical approval was taken from institution review board (IRB), Pokhara Academy of Health sciences with reference no 88/07).

Sample size was calculate from= $Z^2 x(p x q)/e^2$. N= Sample size. Z= Desired reliability (1.96 for 95% CI), P= Estimated proportion in population (17% in this study) Q= 100-P. e= Maximum tolerable error (5%) N= 217. Patients were collected by convenience sampling technique.Written consent was taken from all of the patients prior to the enrollment. Detail history and physical examination were carried out in all of the patients who fulfill the inclusion criteria.Inclusion criteria were any patient of age > 18 years presented with USG report suggestive of fatty liver. Exclusion criteria were any patients who consume more than 30 gm/day for men and >20 gm /day for female, Hepatitis B & Hepatitis C positive status, pregnant lady, any other known liver diseases like autoimmune hepatitis or wilsons diseases, fatty liver with hepatic cysts or features of chronic liver diseases or ascites. Relevant investigation were performed (Complete blood counts, random blood sugar, fasting blood sugar, renal function tests. Prothrombin time, liver function tests, thyroid function tests, fasting lipid profile and hepatitis B surface antigen, Hepatitis C virus antibody and human immunodeficiency virus. A 2 dimensional ultrasound abdomen (Samsung HS 40, Convex 3.5 MHz transducer model CA2-8 AB) was carried out with recording of size, echo texture of liver and grading of fatty liver.Grade O: Normal, Grade I: increasing liver echogenicity (bright liver) as compared with the right renal cortex and spleen, Grade II: Grade I with loss visualization of intrahepatic vascular walls and Grade III: Grade II with impaired visualization of the diaphragm and posterior portion of the right lobe of liver. FibroTouch (F100, Wuxi Hisky Medical Technology Co. Ltd., Wuxi, Jiangsu, China) was performed in all patients using the broadband probe in right 7/8 intercostal space with ten reading and with interquartile range less than 30% and success rate of >60%. Similarly, various score like APRI, FIB 4 score, NAFLD score and BARD score were calculated.With defining the metabolic syndrome according to the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATPIII) criteria total score and individual component were also recorded. The collected data were entered and coded in Microsoft excel 2010 and exported to statistical packages for the social studies (SPSS) version 23 for statistical analysis. The frequency, percentages and mean (SD) were calculated. Spearman correlation coefficient were determine and compare with APRI/NAFLD/BARD and FIB4 score with fibroTouch score. P value of <0.05 were consider as statistically significant.

Results

Among 217 patients were 113 (52.1%) male. Regarding religion,Brahmin/Chhetri compromise 141(65%) followed by Janajati 40 (18.4%) and Dalit 15 (6.9%).

Table 1: Baseline characteristics of patients

Variable	Mean±SD	Range
Age (Years)	48.10±11.62	23-80
Gender	M (113) F(104)	-
BMI(Kg/m2)	32.44±4.82	21.50-45.30
DM	39 (17.97%)	-
Hypothyroidism	32 (14.74%)	-
Hypertension	69(31.8%)	-
Systolic BP(mmHg)	127.3±14.8	90-180
Diastolic BP(mmHg)	81.99±8.7	60-100
Pulse(bpm)	88.8±12.87	58-120
Total Blood count(mm3)	6667.5±12.87	3700-14200
Hemoglobin (gm%)	13.55±1.31	9.80-17.90
Platelets count (mm3)	232545.1±71271	60000-570000
Random blood sugar(mg/dl)	125.3±32.3	78-277
Prothrombin time(sec)	14.60±1.08	12.08-18.00
Creatinine (mg/dl)	0.90±0.17	0.40-1.34
Sodium (mEq/L)	137.2±4.33	129-145
Potassium (mEq/L)	4.07±0.40	3.10-5.20
Total serum bilirubin (mg/dl)	1.01±0.53	0.29-3.90
Direct Billirubin (mg/dl)	0.30±0.23	0.06-1.50
SGOT (U/L)	45. 4±28.5	10-229
SGPT (U/L)	51.7±29.9	11-172
Alkaline phosphatase (IU/L)	123.7±65.1	46-365
Albumin (g/dl)	4.23±0.37	3.70-5.10
Triglyceride (mg/dl)	280.2±131.7	83-1314
HDL (mg/dl)	43.72±10.56	25.87
Cholesterol(mg/dl)	178.4±42.3	79-293

Age distribution demonstrate minimum age 23 years and maximum age 80 yrs. Mean age was 48.10±11.62 years and majority of patients belongs to age group 40-49 years (35.02%) followed by 50-59 years (26.72%).

The most common occupation were house maker 66(30.41%), business 27(12.44%), teacher 23(10.59%), shopkeeper 14(6.45), farmer 12(5.59%), retired soldier 7(3.22%), nursing officer/ foreign employment/driver 5(2.30%) and engineer/banker 4(1.84%).

Majority of the patients were obese on Grade II obesity compromise 140 (64.51%) likewise Grade I obesity 69(31.79%). Minimum BMI was 21.50kg/m2 and maximum BMI was 46.30 kg/m2 and mean BMI was 32.44±4.82 kg/m2 which are shown in table 2.

Table 2: Body mass index distribution of patient N=217

BMI (kg/m2)	Category	Frequency	Max BMI	Min BMI	Mean BMI	SD
<18.5	Under nutrition	0	46.30	21.50	32.44	4.82
18.5-22.99	Normal BMI	1(0.46%)				
23.0-24.99	Overweight	7(3.22%)				
25.0-29.99	Obese I	69(31.79%)				
>30.0	Obesity II	140(64.51%)				

Risk factor for fatty liver were obesity104 (47.92%). Obesity and alcohol 51(23.50%), dyslipidemia and obesity 48 (22.11%) diabetes mellitus and obesity 39(17.97%). Among these patients 110 (50.69%) patients had combination of cause's mainly metabolic syndrome which are shown in table no 3. Among these patients 4 (1.84%) had undetermined etiology.

Table 3: Risk factor for Fatty liver

SN	Risk factor(s)	Frequency	Percentage
1	Obesity	104	47.92
2	Obesity and alcohol(Met ALD)	51	23.50
3	Dyslipidemia and obesity	48	22.11
4	Diabetes Mellitus and obesity	39	17.97
5	Hypothyroidism and obesity	32	14.74
6	Combination (Obesity, alcohol, dyslipidemia and diabetes)	110	50.69
7	Undetermined	4	1.84

Grading of fatty liver by USG abdomen using convex probe were grade I: 25, Grade II: 136 and Grade III: 56. Hepatic steatosis was measured by FibroTouch showing steatosis 0 (<244db/m: 67) steatosis 1 (245-265 db/m: 19), steatosis 2 (266-295 db/m: 32) steatosis 3 (>295 db/m: 99) . On liver stiffness measurement F0-F1 fibrosis (<7.3 kpa: 103), F2 fibrosis (7.3-9.7 kpa: 67), F2-F3 fibrosis (9.8-12.4 kpa): 25), F3-F4 fibrosis (12.5-17.5 kpa: 15) and F4 fibrosis (>17.5 kpa: 7)

Table 4: Ultrasound and Fibrotouch findings of stiffness and hepatic steatosis

UAP (Grading)	Frequency	Liver stiffness (Grading)	Frequency	USG grading of fatty liver	Frequency
<244db/m	67(30.87%)	<7.3 kpa	103(47.46%)	Grade I	25(11.52%)
245-265 db/m	19(8.75%)	7.3-9.7 kpa	67(30.87%)	Grade II	136(62.67%)
266-295db/m	32(14.74%)	9.8-12.4 kpa	25(11.52%)	Grade III	56(25.80%)
>295 db/m	99(45.62%)	12.5- 17.4 kpa	15(6.91%)		
		>17.5 kpa	7(3.22%)		
Minimum(db/m)	178	Minimum (kpa)	3.2		
Maximum(db/m)	376	Maximun (kpa)	23.2		
Mean (db/m)	276±49.16	Mean (kpa)	8.29±3.71		

On measurement of different noninvasive markers of fibrosis mean FIB4 score was 1.44±0.89, mean APRI score 0.55±0.43, mean NAFLD score -1.3±1.6, mean BARD score was 2.26±1.12 and fibrotouch score 8.29±3.71 kpa.

Table 5: Correlation between FIB4, APRI, NAFLD, BARD and fibrotouch score.

Correlation between FIB4, APRI, NAFLD, BARD and Fibrotouch					
Methods	FIB4	APRI	NAFLD	BARD	Fibrotouch
FIB4	1	.649**(<0.001)	.546**(<0.001)	.202**(0.003)	.339**(<0.001)
APRI		1	.515**(<0.001)	0.121(0.073)	.345**(<0.001)
NAFLD			1	.357**(<0.001)	.379**(<0.001)
BARD				1	.263**(<0.001)
Fibro Touch					1

Table 5 presents the Pearson correlation coefficients between five liver fibrosis assessment methods: FIB-4, APRI, NAFLD, BARD, and Fibrotouch. The correlations between each pair of methods were calculated, with corresponding p-values provided to assess statistical significance.

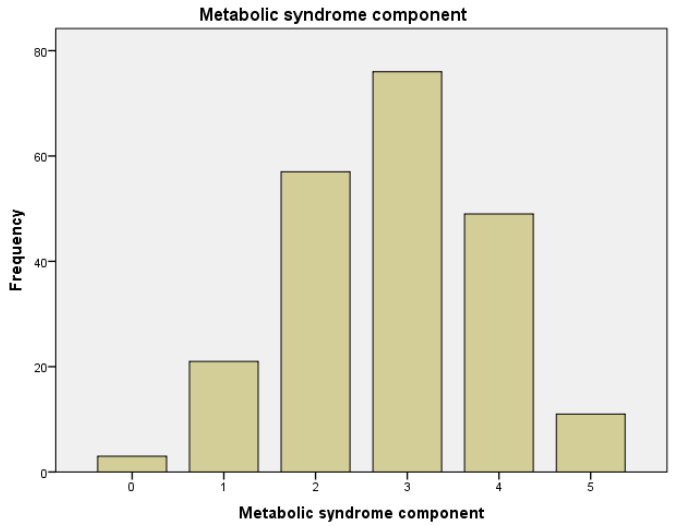

Fig 1: Metabolic syndrome components[6]

Fig 1 showed the component of metabolic syndrome where three components were predominant in fatty liver. Zero component was seen in 3 patients, any one of component (mostly high Triglyceride) was noted in 20(8.9%) patients. Any two components of metabolic syndrome was observed in 57 (25.3%), any three component were in 76(33.8%) and any of four component 50(22.2%) and all component observed in 11(4.9%) patients.

Discussion

NAFLD is commonest cause of chronic liver disease worldwide and are becoming a growing challenge to public health. Prevalence of NAFLD in Nepal varies in different studies. In a retrospective study conducted by Mittal et al in year 2011 showed prevalence of 17.08%.[7] In another study conducted by Shrestha M et al showed prevalence of 38.2% . The overall global prevalence of NAFLD (defined using imaging criteria) is estimated to be 25%.[4] In this study male were the marginally predominant gender (52.1%) while in study conducted by Shrestha M et al[10] showed female preponderance (55.4 %). Regarding age majority of the patients (35.02%) were age group (40-49 yrs) with mean age of 48,10±11.62 years, which is similar to study conducted by Khadgi K and colleagues where mean age was 42.9±12.20 years. Likewise, in this study BMI of >30 kg/m2 consists of 64.70% which is close to study conducted by Khadgi K and colleagues[11] where BMI of 25-30 kg/m2 consists of 61.6% while obesity (>30 kg/m2) was only 16%.

In our study most common association of fatty liver were obesity (47.9%), hypertension(31.8%) hypothyroidism (14.74%), dyslipidemia (22.10%) and diabetes mellitus(17.97%). Most patients had multiple co morbidities association, principally metabolic syndrome. In a study conducted by KC S[12] and colleagues 3 component of metabolic syndrome seen in 73.45% of patients. Study conducted by Kandel et al[13] observed multiple risk factor for NAFLD like, obesity, hypertension, dyslipidemia, diabetes mellitus and genetic factors. Study conducted by Sharma M and colleagues observed prevalence of metabolic syndrome (National cholesterol Elimination Program- Adult Treatment panel III) with all criteria showed 57.6% where as one criteria 91.4%.[14]

At first, USG abdomen was performed for determining degree of hepatic steatosis likewise FibroTouch (F100, Wuxi Hisky Medical Technology Co. Ltd., Wuxi, Jiangsu, China) was used for determing the quantification of hepatic steatosis (Ultrasound attenuation parameter).USG abdomen showed grade II fatty liver was predominant (62.67%) while comparing fibroTouch the ultrasound attenuation parameter (UAP) severe fatty liver >295db/m showed (45.62%).

In a study conducted by Liao Y et al[15] observed BMI, waist, TG, ALT and LSM, but not gender, age, FBG, AST, GGT and hyperuricemia, were the independent risk factors for hepatic steatosis for NAFLD patients which significantly positively affected the degree of hepatic steatosis (P<0.05).

In year 2020 AD metabolic dysfunction associated liver disease (MAFLD) was proposed replacing NAFLD where alcohol was included in any level and later but should have obesity or diabetes mellitus or having two out of seven component of metabolic risk factor. In year 2023 AD newer term metabolic dysfunction associated steotic liver disease (MASLD) was proposed by AASLD by delphi consensus where alcohol drinking was also included, in alcohol drinking category is further divided into MASLD and increased alcohol intake (MetALD) for low and moderate alcohol drinker. MetALD is further divided into Metabolic dysfunction associated steotic liver predominant (MASLD predominant) for those who consume low alcohol <30 gram/day for med and <20 gram/day for female. Similarly, those patient who consume moderate alcohol (<50 gram/day for female and <60 gram/day for male) categories as alcohol predominant (ALD predominant). Those patient who consume >60 gram/day was categories as alcohol related liver diseases (ALD). Likewise, our study included moderate alcohol drinking as AASLD excluding heavy alcohol drinker.

Our study showed obesity and alcohol consumption was second most common risk factor for fatty liver suggesting of multiple pathogenesis or overlapping for causing fatty liver. Most of patients consume locally brewed alcohol or beer of strength 5% w/v. Daily consumptions were <30 gm per day for male and <20 gm for female. Some studies showed that moderate alcohol consumption has beneficial effects in cardiovascular outcomes and prevention of NAFLD.[16] However, recent studies suggest exacerbation of NASH and progression of hepatic fibrosis.[17] Most of these studies are limitation like cross sectional studies, selection bias and unable to quantify alcohol consumption through questionnaire, using noninvasive fibrosis markers without liver histology.[18]

While determining the fibrosis of liver there are various noninvasive markers principally APRI, NAFLD score, BARD score and FIB4 scorewere calculated. FIB4 exhibited the highest correlation with APRI (r = 0.649, p < 0.001), indicating a strong

positive association between these two indices. Moderate correlations were observed between FIB4 and NAFLD ($r = 0.546$, $p < 0.001$), as well as between FIB4 and Fibro Touch ($r = 0.339$, $p < 0.001$). A weaker, but statistically significant, correlation was found between FIB4 and BARD ($r = 0.202$, $p = 0.003$). APRI also demonstrated moderate correlations with NAFLD ($r = 0.515$, $p < 0.001$) and Fibro Touch ($r = 0.345$, $p < 0.001$). However, its correlation with BARD was low and not statistically significant ($r = 0.121$, $p = 0.073$), suggesting limited agreement between these two methods. NAFLD showed moderate positive correlations with both BARD ($r = 0.357$, $p < 0.001$) and Fibro Touch ($r = 0.379$, $p < 0.001$). The correlation between BARD and Fibro Touch was relatively weak but statistically significant ($r = 0.263$, $p < 0.001$). Overall, the results indicate significant correlations among most methods, with the strongest agreement observed between FIB4 and APRI, and weaker relationships between BARD and other methods. These findings highlight the complementary nature of these assessment tools in the evaluation of liver fibrosis, while also suggesting potential differences in their diagnostic focus.

Conclusion

Fatty liver was more prevalent in middle aged male patients. Risk factor are being obesity, Diabetes mellitus, dyslipidemia and alcohol. Three or more component of metabolic syndrome is risk factor for fatty liver. USG abdomen appears first line investigation for fatty liver. Similarly noninvasive tests like FIB4, NAFLD score, APRI score and BARD score are comparable with FibroTouch score.

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