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Original Article

Clinical Profile and Non-invasive Predictors of Fibrosis in a Newly Diagnosed Non-Alcoholic Fatty Liver Disease Patient in a Tertiary Health Care Center of Nepal

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Abstract

Non-Alcoholic Fatty Liver Disease (NAFLD) represents a spectrum of histopathologic abnormalities ranging from simple steatosis to the more aggressive non-alcoholic steatohepatitis, characterized by steatosis, parenchymal inflammation, hepatocellular ballooning and other evidence of hepatic injury. The objective of this study is to evaluate the demographic and anthropometric profile of non-alcoholic fatty liver disease and non-invasive predictors of fibrosis. This is a hospital-based observational study. A total of 280 patients were included in this study from the Department of Gastroenterology, Tribhuvan University Teaching Hospital from January 2019 to August 2021 were included. Patients presenting with non-alcoholic fatty liver disease were mostly (29.64%) of 36-45 age group. Two-third of the patients (66.78%) were asymptomatic. The mean body mass index of patients was 28.10±4.10 kg/m^2 and most of them (81.07%) had body mass index >25 kg/m^2. More than one third of the patients (40%) with non-alcoholic fatty liver disease had presence of metabolic syndrome. The mean (±SD) ultrasound attenuation parameter was 284.22±31.58 dB/m. Fatty liver index showed a positive and a medium strength correlation with fatty liver. On Spearman rank correlation, ultrasound attenuation parameter steatosis grading was correlated positively and strongly with the USG grading of fatty liver (p<0.001). On Pearson correlation, APRI, NFS and FIB-4 were positive but weakly correlated with liver stiffness measurement by FibroTouch but still the correlation was statistically significant (p<0.01). Fatty liver index has shown a good correlation with the presence of steatosis by a FibroTouch. Similarly, APRI showed the highest correlation in predicting liver stiffness in the population of Nepal. Other multicenter large scale prospective analytical studies would be required for further clarify of the results of such a kind of research.

Keywords: *Fatty liver index, fibro touch, non-alcoholic fatty liver disease, ultrasound attenuation parameter.*

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INTRODUCTION

Non-alcoholic fatty liver disease is a common entity which a clinician come across multiple times in their clinical practice. With the rising trend of sedentary lifestyle and unhealthy diet, obesity is in the rise, therefore the incidence of DM has increased since last few decades due to various reasons. All these socio-epidemiological changes have caused the incidence of NAFLD to increase. Globally, NAFLD is gradually overtaking alcoholic liver disease and viral hepatitis as the most common etiology of chronic liver disease. Thus, it has become imperative to define NAFLD/steatosis and the presence of fibrosis in the liver which is the primary driver of progression of liver disease. With the advancement of technology, developed nations have been shifting from the invasive liver biopsy in detecting liver fibrosis and steatosis to detecting liver stiffness by transient elastography. The high cost of this instrument has

precluded the clinician from a developing country like Nepal in having such instruments in their clinic.

Fatty liver is defined as accumulation of lipids within hepatocytes.[1] Fatty liver is a benign and reversible condition and depicts a non-specific response of liver to metabolic stress.[2] In 1980, Ludwig et al described the term non-alcoholic steatohepatitis (NASH) as a form of liver injury that was histologically consistent with alcoholic hepatitis but occurred in obese, diabetic females and who denied alcohol use.[3] The principle concept of the term non-alcoholic fatty liver (NAFL) denotes absence of other causes of fatty liver.[4] Although the diagnosis of NAFL can be made through imaging studies but to diagnose NASH, it requires histology.[5]

Incidence and prevalence of NAFLD is increasing over time mainly due to inappropriate food habit, weight gain, and sedentary lifestyle. Zobair et al. in 2019 showed that the global prevalence of NAFLD in general population is 25% whereas the global prevalence of NASH is 3% to 5%.[6] Accordingly, NASH is considered as the third commonest cause of liver disease after alcohol abuse and viral hepatitis.[7] The prevalence of NAFLD increases to 57.5% to 74% in obese persons and 90% in morbidly obese persons.[5] There is a strong association between occurrence of fatty liver and insulin resistance.[4]

The prevalence of NAFLD in Nepal is not known. Steatosis and fibrosis using vibration controlled transient elastography (VCTE) is done in a few selected centers in Nepal.[8] Thus, this study was intended to assess the clinical profile and non-invasive predictors of liver fibrosis using various scoring systems and transient elastography. This would validate these scoring systems in our population settings and thus could guide clinicians in centers where the more expensive transient elastography is not available.

MATERIALS AND METHODS

The study was carried out in the Department of Gastroenterology at Tribhuvan University, Institute of Medicine (TU, IOM), Maharajgunj, Kathmandu, Nepal for 1 year. This was a quantitative observational cross-sectional study. Non-probability consecutive sampling method was used in this study since all patients meeting the inclusion criteria were included in the study until the desired sample size was met. A sample size of 280 was estimated based on the sample size formula: $Z^2*P\ (1-P)/d^2$ where n = sample size, Z = Z statistics for level of significance, P = expected prevalence or proportion and d = precision. Sample size was calculated using a prevalence (P) of NAFLD of 24% with a 95% confidence interval (Z=1.96) and a precision(d) of 5%. The inclusion criteria were: patients with obesity, metabolic syndrome or diabetes, patients diagnosed with steatosis in USG incidentally, age > 16 years, those who provided informed consent. The exclusion criteria were: patients known to have significant alcohol history (>20 g/day in females and 30 g/day in males), taking drugs causing fatty liver and other hepatotoxic drugs, hepatitis B and C, autoimmune hepatitis (positive ANA), Wilson's disease, cardiac failure, pregnant woman, age < 16 years, those who did not provide informed consent.

Demographic variables like ethnicity, age, gender, diet, activity and clinical variables like chief complaints, significant examination findings, comorbid conditions if any were recorded. Laboratory variables like complete blood count, liver function test, fasting lipid profile, thyroid function tests, kidney function test, uric acid, viral markers, ANA and ferritin were assessed. USG was done by Philips iU22 USG machine. UAP was assessed by FibroTouch VCTE (Kerry Medical Limited). Various scores were calculated and correlated with the results of transient elastography.

RESULTS

A total of 280 patients with NAFLD were included; Here 112 (40%) patients with NAFLD had metabolic syndrome, while only 25 (8.92%) had syndrome Z. On reviewing the age, the mean age of patients was 44.94±11.99 years; 83, (29.64%) patients presenting with NAFLD were in age group of 36-45 years. Among the study population 164 (58.57%) were males as compared to females 116 (41.43%). Most of the patients were evaluated for NAFLD due to incidental detection of fatty liver in USG or asymptomatic elevation in transaminases done during routine investigation or done for evaluating other diseases.

Table 1 shows various anthropometric characteristics of the patients. The mean BMI was 28.10±4.16 (min: 19, max: 45) and 227 (81.07%) of the patients had BMI >25 kg/m^2. The mean waist circumference was 91.97±6.87 cm, whereas the mean for men and women were 91.05±4.83 cm and 93.97±8.83 cm respectively.

Table- I: Anthropometric Characteristics of Patients

Characteristics	Frequency (Percentage) /Mean ± SD (Min, Max)
Age (years)	44.94±11.99 (23, 82)
BMI (kg/m^2)	28.10±4.16 (19,45)
BMI >25 (kg/m^2)	227 (81.07%)
Waist circumference (cm)	
Male	91.05±4.83
Female	93.27±8.83
Total	91.97±6.87 (78, 122)
Hip circumference (cm)	
Male	96.47±5.88
Female	99.51±9.14
Total	97.73±7.56 (83, 132)
Mid-upper arm circumference (cm)	26.83±2.98 (22, 36)
Triceps skin fold thickness (mm)	15.58±3.62 (8,35)
Waist hip ratio	
Male	0.94±0.06
Female	0.93±0.02
Total	0.94±0.04

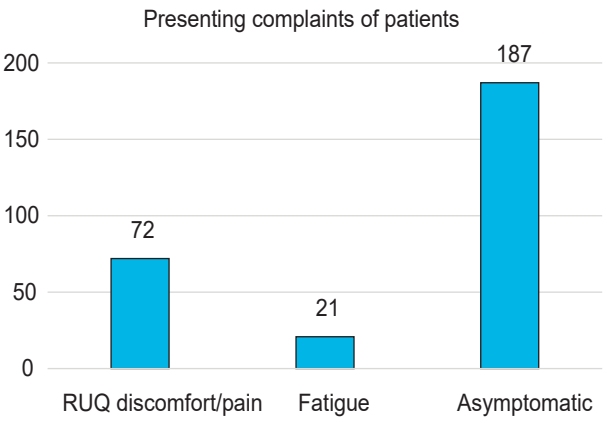

Figure- 1: Presenting Complaints of Patients

Figure 1 depict the presenting complaints of patients; here presenting complaint of right upper quadrant (RUQ) discomfort was 72 (25.71%) and fatigue was 21 (25%) among the symptomatic patients. Asymptomatic patients were 187 (66.79%).

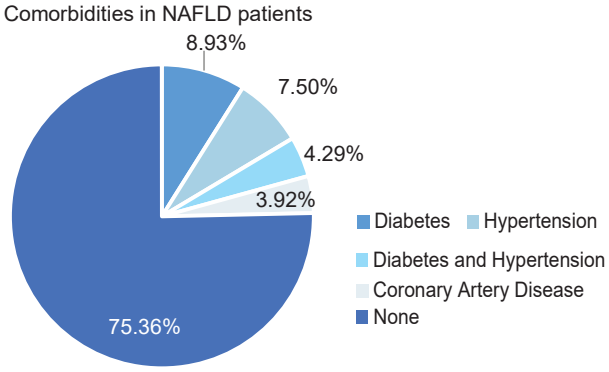

Figure- 2: Comorbidities in NAFLD Patients

Figure 2 states the distribution of comorbidities in NAFLD patients; here 75.36% had no comorbidities, where diabetes mellitus, hypertension, both diabetes and hypertension, coronary artery disease were found in 8.93%, 7.50%, 4.29% and 3.93% respectively.

Table II contains the distribution of baseline laboratory parameters of the patients. Here Mean±SD with range (minimum, maximum) of fasting lipid profile, liver function test, total protein, albumin, uric acid and fasting blood glucose were measured. The Mean±SD with range (minimum, maximum) were total cholesterol 4.10±2.87 mmol/L (2, 38), HDL 1.10±0.42 mmol/L (0.4, 4), LDL 2.27±0.90 mmol/L (0.5, 5), Triglyceride 2.68±2.11 mmol/L (0.8, 28), Total bilirubin 15.12±8.62 mmol/L (4, 66), Direct bilirubin 3.90±3.77 µmol/L (1, 47), AST 47.88±46.71 IU/L (10, 635), ALT 63.38±56.41 IU/L (10, 518), GGT 54.40±37.07 IU/L (10, 269), ALP 132.88±87.41 IU/L (38, 573), Total protein 73.31±6.43 gm/L (54, 91), Albumin 43.17±5.31 gm/L (22, 61), Uric acid 294.49±71.49 umol/L (150, 600) and Fasting blood glucose 5.33±1.78 mmol/L (3, 21).

Table- II: Baseline Laboratory Parameters of the Patients

Characteristics	Mean ± SD (Min, Max)
Total cholesterol (mmol/L)	4.10±2.87 (2, 38)
HDL cholesterol (mmol/L)	1.10±0.42 (0.4, 4)
LDL cholesterol (mmol/L)	2.27±0.90 (0.5, 5)
Triglyceride (mmol/L)	2.68±2.11 (0.8, 28)
Total bilirubin (µmol/L)	15.12±8.62 (4, 66)
Direct bilirubin (µmol/L)	3.90±3.77 (1, 47)
AST (IU/L)	47.88±46.71 (10, 635)
ALT (IU/L)	63.38±56.41 (10, 518)
GGT (IU/L)	54.40±37.07 (10, 269)
ALP (IU/L)	132.88±87.41 (38, 573)
Total protein (gm/L)	73.31±6.43 (54, 91)
Albumin (gm/L)	43.17±5.31 (22, 61)
Uric acid (umol/L)	294.49±71.49 (150, 600)
Fasting blood glucose (mmol/L)	5.33±1.78 (3, 21)

Table III refers to the ultrasonography grading of fatty liver and FibroTouch findings of the patients; the mean (±SD) UAP was 284.22±31.58 dB/m, but 7.1% of the patients diagnosed to have NAFLD by USG had no steatosis (S0) during UAP evaluation by FibroTouch. On Spearman rank correlation, UAP steatosis grading was correlated positively and strongly with the USG grading of fatty liver (spearman rank correlation 0.653, p<0.01).

Table-3: Ultrasonography grading of fatty liver and FibroTouch findings of the patients

Characteristics	Categories (Percentage)	Frequency
USG grading of fatty liver	1	96 (34.3%)
	2	116 (41.4%)
	3	68 (24.3%)
Steatosis grading by UAP	S0	20 (7.1%)
	S1	37 (13.2%)
	S2	108 (38.6%)
	S3	115 (41.1%)
UAP (Mean±SD {Min, Max})	284.22±31.58 (191, 400)	
Fibrosis grading by LSM	F0-F1	128 (45.7%)
	F2	81 (28.9%)
	F3	49 (17.5%)
	F4	22 (7.9%)
LSM (Mean±SD {Min, Max})	7.98±2.92 (3, 18)	

Table IV shows Pearson's correlation coefficient and the p-value for each of correlations which depict that weight and BMI are positively and largely correlated with UAP value and the correlation is statistically significant (p<0.01). Waist circumference was positively and had medium correlation with UAP value and the correlation is statistically significant (p<0.01). Metabolic syndrome and ALT are positively but small correlation with UAP value, but the correlation was statistically significant (p<0.01). Although, total cholesterol, LDL and fasting blood glucose had positive and small correlation with UAP but it was statistically insignificant.

Table 4. Correlation of UAP Values of FibroTouch with Other Covariates

Covariates	Pearson Correlation	P-value
Age	-0.072	0.23
Weight	0.51	<0.01
Waist circumference (cm)	0.413	<0.01
BMI	0.572	<0.01
Metabolic syndrome	0.231	<0.01
Total cholesterol (mmol/L)	0.105	0.078
HDL cholesterol (mmol/L)	-0.143	0.017
LDL cholesterol (mmol/L)	0.107	0.074
Triglyceride (mmol/L)	-0.004	0.948
ALT (IU/L)	0.219	<0.01
Fasting blood glucose (mmol/L)	0.038	0.522

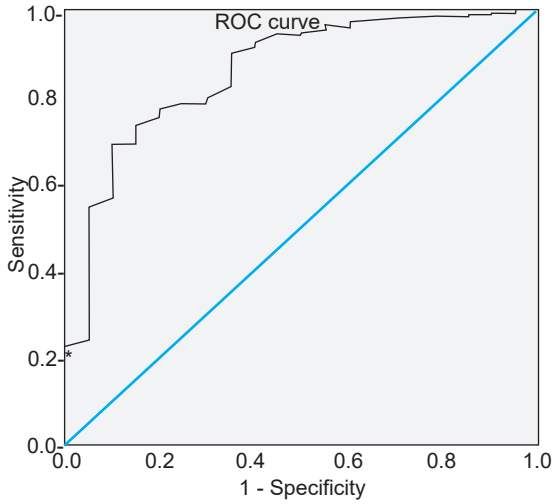

Figure- 3: ROC curve showing a significant area under curve (AUC) of FLI

Figure 3 demonstrates the Receiver Operating Characteristic (ROC) curve; here, ROC curve shows a significant area under curve (AUC) of 0.859. Fatty liver index (FLI) showed a positive but a medium strength correlation with fatty liver as shown by FibroTouch (correlation coefficient value 0.381, p-value <0.01).

Table 5 describes the correlation of non-invasive predictors of fibrosis and stiffness shown by FibroTouch; on pearson correlation, APRI, NFS and FIB-4 were positively but weakly correlated with LSM by FibroTouch but still the

correlation was statistically significant (p<0.01). BARD was positively correlated but it was statistically insignificant.

Table- 5: Correlation of non-invasive predictors of fibrosis and stiffness shown by FibroTouch

Covariates	Pearson Correlation	AUC	P-value
APRI	0.194	0.594	<0.01
NFS	0.212	0.536	<0.01
FIB-4	0.267	0.578	<0.01
BARD	0.009	0.490	0.883
Metabolic syndrome	-0.02		0.739

DISCUSSION

Nonalcoholic fatty liver disease represents a major public health concern. It is associated with type 2 diabetes, metabolic syndrome, and other cardiovascular risk factors, and may lead to fibrosis, cirrhosis, liver cancer, liver failure requiring liver transplant, and mortality. A total of two hundred eighty patients with NAFLD were included in the study. On reviewing the age, patients presenting with NAFLD were mostly of 36-45 age group (N=83, 29.64%). The mean age was 44.94±11.99 (min: 23, max: 82).

Most of the study population were males (N=164, 58.57%) as compared to females (N=116, 41.43%). This pattern of distribution of NAFLD in females is believed to be due to alteration in sex hormone levels, specifically reduced estrogens and increased androgens during and after menopause. Most of the patients were evaluated for NAFLD due to incidental detection of fatty liver in USG or asymptomatic elevation in transaminases done during routine investigation or done for evaluating other diseases which was similar to the findings shown by Joel Z. Stengel et al.[9] Right upper quadrant (RUQ) discomfort was the most common presenting complaint among the symptomatic patients which was on accordance with study done by Metin Basaranoglu et al.[10] Most of the patients did not have any comorbidities. Diabetes mellitus was the most common comorbidity present in these patients. More than one third of the patients with NAFLD had presence of metabolic syndrome (N=112, 40%). Similar finding was shown by Raxit Kumar Jinjuvadia et al. in a study evaluating 11,674 United States population. The mean BMI was 28.10±4.16 (min: 19, max: 45) which was similar to the study done by Sadroddin Lahsaee et al.[11] 227 (81.07%) of the patients had BMI >25 kg/m^2 which implied that more than three forth of patients with NAFLD were obese. 9.29% of patients had lean NAFLD. The mean waist circumference was 91.97±6.87 cm, whereas the mean for men and women were 91.05±4.83 cm and 93.97±8.83 cm respectively which shows that majority of patients had waist circumference higher than the cutoff indicated for Asian population by International Diabetes Federation.

This study showed fatty liver index (FLI) had a positive but a medium strength correlation with fatty liver as shown by FibroTouch (correlation coefficient value 0.381, p-value <0.01). This depicted a good strength in predicting the presence of fatty liver without using FibroTouch which could be of great benefit in a resource limited country like ours. Receiver operating characteristic (ROC) curve shows a significant area under curve (AUC) of 0.859.

Since the transient elastography instrument is costly, it is not available widely in a resource limited country like Nepal. In addition, liver biopsy for evaluating the presence of liver fibrosis is not practical in all patients. Thus, non-invasive predictors for detecting and grading liver fibrosis is of paramount importance. Various non-invasive predictors have been developed. This study highlights the correlation of these predictors with LSM value given by elastography. On Pearson correlation, APRI, NFS and FIB-4 were positively but weakly correlated with LSM by FibroTouch but still the correlation was statistically significant (p<0.01) in this study. Whereas, BARD was positively correlated but it was statistically insignificant (p=0.883). Area under the ROC curve (AUROCs) of APRI, NFS, FIB-4 and BARD score were 0.594, 0.536, 0.578 and 0.490 respectively. Despite these predictors having positive correlation, APRI, NFS and FIB-4 had weak correlation and BARD had statistically insignificant correlation.

In a study done by Alhankawi, Dhuha et al. FIB-4 and APRI correlated significantly with fibroscan score (r=0.472, p<0.0001 and r=0.418, p<0.0001).[12] The aforementioned study also showed an AUROCs of FIB-4 and APRI were 0.705 and 0.644 respectively.[12] Similarly, study done by Sebastian Zenovia et al. revealed a significant correlation between LSM values and APRI (r = 0.19, p = 0.020), FIB-4 index (r = 0.34, p < 0.001), and NFS (r = 0.30 p < 0.001).[13] Study done by Savvoula Savvidou et al. in Greek Tertiary Liver Centers showed a positive correlation of BARD score with LSM with

AUROC of 0.724±0.041 (95% CI 0.645-0.804), p<0.001.[14] Our study revealed that APRI had a higher accuracy for the prediction of significant fibrosis followed by FIB-4 and NFS. BARD score had the least accuracy in predicting fibrosis.

CONCLUSION

The role of non-invasive predictors arises which predict the presence of steatosis and fibrosis by using various biomarkers. Although, these non-invasive predictors have been tested in other countries, they have not been studied in the population of Nepal. Thus, the aim of this study was to evaluate the accuracy of these non-invasive predictors and its correlation with FibroTouch. Fatty liver index has shown a good correlation with the presence of steatosis by a FibroTouch. Similarly, APRI has shown the highest correlation in predicting liver stiffness in the population of Nepal. A good correlation was also seen with FIB-4 and NFS but BARD score showed the least accuracy. Other multicenter large prospective studies are required to further clarify the results of such a kind of research.

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