Original Article

VALIDATION OF FATTY LIVER INDEX FOR NONALCOHOLIC FATTY LIVER DISEASE IN PAKISTANI ADULTS

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ABSTRACT

Objective: To compare the diagnostic accuracy of fatty liver index (FLI) with abdominal ultrasonography (USG) for the nonalcoholic fatty liver disease.

Study design: Cross sectional analytical study.

Place & duration of study: PNS SHIFA hospital Karachi, from August 2015 to July 2016.

Materials and Methods: Adults of either gender aged more than 20 years who reported to radiology department for USG abdomen were consecutively inducted. Patients with diabetes mellitus, hypertension, chronic liver disease, those with significant alcohol intake and taking lipid lowering drugs were excluded. Anthropometric and biochemical data were collected by a standard protocol. NAFLD was diagnosed by hepatic USG. FLI being an index test was compared with USG taken as reference standard. The accuracy and cut-off point of the FLI to detect NAFLD were evaluated by area under the receiver operator characteristic curve (AUC) and the maximum Youden index analysis, respectively.

Results: NAFLD was present in 72(34%) out of 210 subjects. The AUC of the FLI for NAFLD was 0.876 (95% confidence interval: 0.818–0.916), and larger than that of its each individual component [0.787 (0.722–0.853), 0.739 (0.661–0.816), 0.754 (0.689–0.82), and 0.774 (0.706–0.841) for waist circumference (WC), body mass index (BMI), triglyceride (TG), and γ-glutamyl transferase (GGT), respectively] (all P =0.000). The optimal cut-off point of the FLI for diagnosing NAFLD was 30 with the maximum Youden Index of 0.537, achieving a high sensitivity of 80.55% and a specificity of 73.19%.

Conclusion: The FLI could accurately identify NAFLD at optimal cut-off point of 30 in Pakistani adults.

Keywords: NAFLD, Fatty liver index, Ultrasound, Youden index, GGT, BMI.

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INTRODUCTION

Non-alcoholic fatty liver disease (FLD) is defined as macrovesicular steatosis in hepatocytes in the absence of alcohol use and any other obvious damaging factor. NAFLD is the most eminent cause of chronic liver disease in western world that has affected 46% of adults [1]. NAFLD includes a complete spectrum of hepatic pathologies ranging from simple steatosis to nonalcoholic steatohepatitis (NASH) that may leads to cirrhosis and finally hepatocellular carcinoma [2]. The prevalence of NAFLD has doubled during last 20 years due to the current epidemic of obesity and its complications especially hypertriglyceridemia, insulin resistance and type 2 diabetes mellitus [3]. It may represent another component of metabolic syndrome (MetS)[4]. Accumulating evidence from recent studies has suggested that NAFLD may play an imperative role in the progression of cardiovascular disease and chronic kidney disease [5,6]. Therefore, early detection of NAFLD is necessary to halt the disease progression and its extrahepatic manifestations.

NAFLD can be diagnosed either by imaging or by histology and absence of secondary hepatic fat accumulation [2]. Liver biopsy is the reference standard technique to identify NAFLD and to establish the diagnosis of NASH [7]. But it is not possible to perform this painful and invasive procedure with low but definite risk of life threatening bleeding in a large number of patients [4,7,8]. Considering occult nature of the disease, a simple, cost-effective and preferably quantitative diagnostic method would be useful for early detection and better management of NAFLD patients [9]. The commonest noninvasive method for the qualitative assessment of

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fatty liver is Abdominal USG that can detect moderate to severe hepatic steatosis with a reasonable sensitivity and specificity. However, its limitations include high interobserver variability, difficulty in obese patients who obviously are in high proportion in NAFLD [10]. Clinical risk factors, such as the components of the MetS, as well as emerging biomarkers can help select NAFLD patients. The "fatty Liver index" (FLI) is a simple but accurate surrogate steatosis biomarker. The algorithm of FLI was based on four potential predictors of FL namely WC, BMI, TG and GGT and can be used to predict hepatic steatosis in general population [11]. FLI was a very convenient marker as its individual components are routinely measured in clinical practice [1,4].

FLI has been proved as a practical, reliable, and cost-effective method to diagnose NAFLD in large epidemiology studies in several countries [1,12,13]. However, due to variation of ethnicity, dietary and environmental factors, the cut-off for waist and BMI is different for the Asian people[14]. Therefore, FLI needs to be validated when used in a different population. The present study aimed to validate the accuracy and the optimal cut-off point of the FLI for diagnosis of NAFLD in Pakistani adults.

MATERIALS AND METHODS

This hospital based cross sectional study was conducted in the department of radiology and chemical pathology, PNS SHIFA hospital Karachi from August 2015 to July 2016 after approval by the Institutional review board. The sample size of 210 was estimated by using Sensitivity & specificity sample size calculator of Lin Naing taking 80% sensitivity and 72% specificity of FLI against US [1], 14% local prevalence of NAFLD [15,16] while keeping error probability at 0.05 and statistical power at 0.85.

Individuals of either gender, aged more than 18 years were considered for further inclusion in the study. Those with history of significant alcohol intake (more than 30 g/d in males and more than 20 g/d in females), hepatitis, chronic liver disease, any other chronic ailment and use of lipid lowering drugs were excluded. Eventually, a total of 210 subjects were finally selected for the study. After informed consent all participant underwent a detailed clinical and anthropometric evaluation including gender, age, WC, weight and height. BMI was calculated by using the formula [weight (kg)/height (meter^2)]. Venous samples after 12 hours fast were analyzed by standard laboratory procedures using spectrophotometric technique for GGT, total cholesterol (TC), HDL cholesterol (HDL-C), TG, LDL cholesterol (LDL-C), plasma fasting glucose (PFG) and glycated hemoglobin (HbA1c). Non-HDL cholesterol (NonHDL-C) was calculated by subtracting HDL-C from TC. Serum insulin levels were measured using electrochemiluminescence technique on Cobas e411 immunoassay analyzer. Insulin resistance (IR) was determined by the homeostasis model assessment (HOMAIR) method using the following formula: HOMA-IR = [PFG (mmol/l) × fasting insulin (µUI/ml)]/22.5. IR was defined as HOMA-IR ≥ 2.25. FLI was calculated by using the following formula:

$$FLI=\left[\frac{e^{0.953\times \ln(TG)+0.139\times BMI+0.718\times \ln(GGT)+0.053\times WC-15.745}\times 100}{\left(1+e^{0.953\times \ln(TG)+0.139\times BMI+0.718\times \ln(GGT)+0.053\times WC-15.745}\right)}\right]$$

The hepatic USG was performed in all patients after 12 hours fast by a single experienced radiologist on GE Logic C5 premium using 5 MHz probe. The hepatic steatosis was diagnosed using well-established criteria, including the hepatorenal echo contrast, liver parenchymal brightness, deep beam attenuation and vascular blurring. NAFLD was determined via evidence of hepatic steatosis in the USG and a lack of evidence of other causes of acute or chronic hepatitis.

All data including demographic and biochemical parameters was analyzed by Statistical Package for Social Sciences version 20 (SPSS Inc, Chicago, IL, USA). All data was checked for normality using Kolmogorov-Smirnov test. Categorical variables were presented as frequency in percentages. Continuous variables were presented as Mean ± SD for parametric variables or median (interquartile range) for skewed variables. Area under the receiver operator characteristic curve (AUC) was used to show the predictive value of FLI for diagnosing NAFLD. The diagnostic accuracy parameters like sensitivity (Sn), specificity (Sp), predictive values, likelihood ratios and Youden index were calculated at various FLI cut offs and the point with the maximum Youden index was used as the optimum value of the FLI for detecting NAFLD. All the participants were group according to the presence of NAFLD-FLI and their demographic and biochemical characteristics were compared using independent sample t test, Mann-Whitney U test or Fisher's exact test for continuous parametric, skewed and categorical variable respectively. A "*p*" value of < 0.05 was considered significant.

RESULTS

Out of 210 participants, 115(55%) were females and 95 (45%) were males. The mean age of all participants was 46.8±12.16 years. 72 participants had ultrasonographically defined NAFLD. Table-1 compares demographic and biochemical characteristics of the participants with and without NAFLD on USG. Though no significant difference was found in mean age and gender between two groups; subjects with NAFLD had higher levels of BMI, FPG, serum insulin, HOMA IR, HbA1c, TC, TG, LDL-C, HDL-C, NonHDL-C, GGT and ALT (all *P* < 0.005). FLI was also significantly high in NAFLD patients compared with non-NAFLD participants (*p*=0.000).

As shown in Table-2, univariate logistic regression analysis revealed significant association of HbA1c, ALT, HOMA-IR, non-HDL-C and FLI with an increased risk of NAFLD. However, after adjusting the effects of all these predicting variables in the multivariate model, FLI was still significantly associated with NAFLD (adjusted OR: 1.066; 95% CI: 1.032–1.1, P =0.000).

The diagnostic accuracy of FLI for NAFLD, as predicted by US abdomen, yielded an AUC of 0.867 (95% CI: 0.818-0.916) (figure-1). The predictive performance of each individual component of FLI was significantly lower than that of FLI as shown in figure-1.

Table-3 compared diagnostic performance characteristics for 10 unit intervals of FLI. It revealed that FLI< 30 can be used as optimal cutoff value to rule out NAFLD with the maximum Youden index of 0.537 and (sensitivity = 80.55%; specificity = 73.19%; PPV= 61.05%; NPV = 87.83%, LR+= 3.007, LR-= 0.285). On the other hand, A FLI > 50 showed better specificity and PPV; hence can be used to rule in NAFLD (sensitivity = 51.39%; specificity = 97.1%; PPV = 90.24%; NPV= 79.29%, LR+= 17.13, LR-= 0.501, Youden index = 0.484).

Table-1: Demographic and biochemical characteristics of study population.

Variable	Overall	Non-NAFLD	NAFLD	*p*-value
n(%)	210	138(66)	72(34)	
Male, n(%) [$]	95 (45)	56 (59)	39 (41)	0.330

Age, years @	46.8 ± 12.16	47.7 ± 10.88	46.13 ± 13.13	0.361
Waist circumference, cm #	85(82-91.3)	82.5 (79.4-85)	91.7 (85.5-95.4)	0.000*
BMI, kg/m^2 #	23.2 (21.8-24.9)	22(21.4-23.4)	25.2 (23-27.8)	0.000*
FPG, mmol/L #	5.5(4.9-6.2)	5.3(4.8-6.0)	6.1(5.3-6.6)	0.002*
Serum Insulin, mIU/L#	6.95(5.5-12.1)	6.0(5-7.4)	14.1(8.6-20.3)	0.000*
HOMA IR #	1.64(1.34-2.68)	1.42(1.23-1.66)	3.59(2.13-5.6)	0.000*
HbA1c(%) #	5.5(5.0-6.2)	5.3(4.8-5.9)	6.1(5.3-6.6)	0.000*
TC, mmol/L #	4.5(3.8-5.1)	4.2(3.8-4.8)	4.95(4.4-5.6)	0.000*
TG, mmol/L #	1.58(1.19-2.1)	1.24(1.0-1.74)	2.0(1.59-2.6)	0.000*
LDL-C, mmol/L #	2.89(2.2-3.32)	2.7(2.1-3.05)	3.1(2.26-3.7)	0.002*
HDL-C, mmol/L #	1.0(0.9-1.1)	1.03(0.92-1.1)	0.93(0.88-1.0)	0.000*
NonHDL-C, mmol/L #	3.4(2.8-4.0)	3.1(2.59-3.84)	3.9(3.3-4.66)	0.000*
GGT, U/L #	28(23-32)	25(22-29)	32(28-37.8)	0.000*
ALT, U/L#	20(14-26)	17(13-23)	27(20-37)	0.000*
FLI#	28.5(19-42)	23(15-30.25)	51.5(32-66.75)	0.000*

** significant; @ Mean±SD; compared using independent samples t-test, $ proportions; compared using Fisher exact test, # median and range; compared using Mann Whitney U-test, ALT= alanine transaminase, BMI= body mass index, FLI= fatty liver index, FPG= fasting plasma glucose, GGT= gamma-glutamyl transferase, HbA1c= glycated hemoglobin, HDL-C= high-density lipoprotein cholesterol, HOMA-IR= homeostasis model assessment for insulin resistance, LDL-C=low-density lipoprotein cholesterol, TG= triglycerides, TC= Total cholesterol.*

Table-2: Results of univariate and multivariate logistic regression analysis of factors associated with NAFLD.

Variables	**Univariate logistic regression**			**Multivariate logistic regression**		
	Wald test	***P* value**	**OR (CI)**	**Wald test**	***P* value**	**OR (CI)**
FLI	48.46	0.000	1.099(1.070-1.129)	15.465	0.000	1.066(1.032-1.1)
ALT	31.843	0.000	1.114(1.073-1.157)	2.997	0.083	1.047(.994-1.102)
Non-HDL-C	24.692	0.000	2.659(1.808-3.91)	0.445	0.505	1.427(0.502-4.054)
HOMAIR	38.941	0.000	3.619(2.416-5.42)	9.917	0.002	2.143(1.333-3.443)
HbA1c	23.239	0.000	2.765(1.829-4.182)	0.004	0.951	1.017(593-1.744)

FLI= fatty liver index, ALT= alanine transaminase, nonHDL-C= non-high-density lipoprotein cholesterol, HOMA-IR= homeostasis model assessment for insulin resistance, HbA1c= glycated hemoglobin, OR= odds ratio, CI= confidence interval

Table-3: Diagnostic performance characteristics of Fatty liver index (FLI) at different cut-offs.

FLI	**Sensitivity**	**Specificity**	**PPV**	**NPV**	**LR+**	**LR-**	**Youden index**
20	97.22	40.58	46.05	96.55	1.636	0.069	0.378
30	80.55	73.19	61.05	87.83	3.007	0.265	**0.537**
40	63.89	86.23	70.77	82.07	4.63	0.419	0.501
50	51.39	97.1	90.24	79.29	17.13	0.501	0.484
60	34.72	97.82	89.28	74.17	15.77	0.667	0.318
70	20.83	98.55	88.23	70.46	14.85	0.803	0.193

PPV= positive predictive value, NPV= Negative predictive value, LR+= positive likelihood ratio, LR-= Negative likelihood ratio

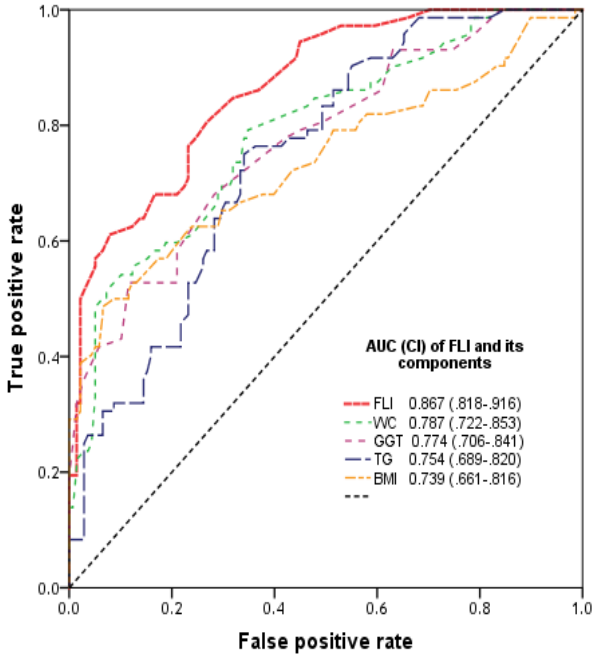

Figure-1: ROC analysis of fatty liver index (FLI), waist circumference (WC), serum γ-glutamyl transferase (GGT), serum triglycerides (TG) and body mass index (BMI) for the diagnosis of nonalcoholic fatty liver disease keeping diagnosis through abdominal ultrasound as reference standard.

DISCUSSION

NAFLD, being the most prevalent chronic liver disease and an independent risk factor for type 2 DM and cardiovascular disease, has considerable implications on public health and economy [17,18,19]. The occult nature of the disease also makes its diagnosis difficult at early stage [20]. So, the development of a simple, cost effective and preferably quantitative screening method is important for early detection of NAFLD. The "fatty Liver index" (FLI) is a surrogate steatosis biomarker developed using patient data of the Dionysos Nutrition & steatosis. 13 variables were evaluated in patients with ultrasound diagnosed fatty liver and those without suspected liver disease. Four variables namely WC, BMI, TG and GGT were selected as potential predictors of FL at bootstrapped stepwise logistic regression analysis. Based on these variables, the algorithm of FLI was formulated to predict hepatic steatosis in general population [11].

The present study revealed the strong discriminatory power of FLI in diagnosing NAFLD in our setup. We found that FLI could accurately detect NAFLD with a good AUC of 0.867 (0.818–0.916) and the optimal cut-off point of the FLI for diagnosing NAFLD was 30 with high sensitivity of 80.55%, specificity of 73.19% and maximum Youden index of 0.537. An independent strong association between NAFLD and FLI was also confirmed by multivariate logistic regression, to the point that a one-unit increase in FLI led to a 6.6% increase in the risk of developing NAFLD. FLI was first proposed and validated by Bedogni et al in 2006 and comprised of TG, BMI, GGT and WC to predict NAFLD in Italian population with a good AUC of 0.84 (95% CI: 0.81–0.87) keeping USG abdomen as reference standard [11]. FLI has also been validated in some other populations with varying results. Koehler et al in his study on elderly white persons of Rotterdam revealed an AUC of 0.807 for FLI in patients with fatty liver and 0.813 for NAFLD. However, the sensitivity of the recommended cut-off of the FLI <30 to rule out fatty liver was comparably lower, probably due to elder study population [15]. Two studies on Caucasian population by Carvalhana et al (2013) and Meffert et al (2014) has also reported satisfactory performance of FLI for diagnosing NAFLD with AUC of 0.930 and 0.890 respectively [21,22]. The first study to validate FLI in Asian population by Kim et al (2011) in Korea showed that FLI was a useful index for predicting fatty liver but it was not superior to WC and BMI [23]. It may be attributable to the small sample size and higher GGT and TG levels in the study population. Recently a population based study by Huang et al on Chinese adults has provided strong evidence on discriminatory power of FLI to diagnose NAFLD (AUC: 0.834) with an optimum cut off point of 30 to rule out the disease[1]. These results are in concordance with findings of our study. Though all the studies has validated FLI against USG abdomen which itself has low sensitivity to detect mild steatosis

and has high interobserver variability, a recent comparison with magnetic resonance spectroscopy (MRS) has shown good accuracy of FLI to detect steatosis [24].

There are certain limitations in our study. First, USG was used to validate FLI as liver biopsy could not be obtained in a screening study for NAFLD. Second, lack of information on the severity of hepatic steatosis has restrained us from finding specific cutoffs for steatosis quantification.

CONCLUSION

FLI can accurately diagnose NAFLD at optimal cut-off point of 30 units in Pakistani adults.

AUTHORS CONTRIBUTION

Qurrat-ul-Ain: Entire research work, sample collection, data analysis, write up.

Atif Latif: Ultrasonographic assessment, literature review.

Syed Raza Jaffar: Arrangement of reagent kits, overall supervision.

Maliha Saleem: Help in sample collection and ultrasonographic assessment.

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