The Egyptian Journal of Hospital Medicine (January 2023) Vol. 90 (2), Page 2108-2113

Efficacy of Ultrasonography and Fibroblast Growth Factor 21 for Early Diagnosis of Non-Alcoholic Fatty Liver Disease

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ABSTRACT

Background: Non-alcoholic fatty liver disease (NAFLD) is a worldwide public health problem. It ranges from simple steatosis to steatohepatitis, liver cirrhosis and hepatocellular carcinoma (HCC). Ultrasonography is a noninvasive tool to examine the liver parenchyma. The aim of the current study is to evaluate the accuracy of ultrasonography in combination with fibroblast growth factor 21 (FGF21) in early detection and diagnosis of NAFLD, especially early stages (mild NAFLD).

Patients and methods: A total of 340 eligible obese subjects were included in the study. They performed ultrasonographic examination with serum levels of FGF21 together with fasting blood glucose, insulin, glycated hemoglobin, total lipid profile, liver function tests and HOMA-IR.

Results: There were significant differences in ultrasonographic findings and FGF21 serum levels between NAFLD group and non-NAFLD group. Roc curve showed a significant area under curve with a significant cutoff for FGF21 serum levels (>160 pg/mL) and intrahepatic triglyceride content (IHTC) (>5.79%). The sensitivity was 83.3% for FGF21 serum levels and 87.8% for IHTC and the specificity was 80% for FGF21 serum levels and 85% for IHTC. After combination of both FGF21 serum levels and IHTC, the sensitivity increased to 88.3% and the specificity was 80%.

Conclusion: The combined use of ultrasonography and serum FGF21 has a higher sensitivity in the early diagnosis of NAFLD compared to either method alone.

Keywords: Nonalcoholic fatty liver disease, FGF21, Ultrasonography, Comparative study, Diagnostic Test.

INTRODUCTION

Nonalcoholic liver disease (NAFLD) is the most common worldwide emerging public health problem representing approximately 25% of the general population. It ranges from benign simple steatosis to malignant steatohepatitis, liver cirrhosis and hepatocellular carcinoma (HCC) [1].

Hepatic steatosis is graded as minimal (< 5%), mild (5%-33%), moderate (33- 66%), and severe (≥ 66%) [2]. Owing to the nonspecific presentation and mild symptoms of NAFLD, early diagnosis and search workup is mandatory to avoid steatohepatitis. Liver biopsy is the gold standard test to diagnose NAFLD. However, its invasive nature and missed interpretation made biopsy alternative measures are introduced to avoid its drawbacks [3].

Ultrasonography is a noninvasive tool to examine the liver parenchyma. Ultrasonography divided the liver into bright echo liver, increased hepato-renal echogenicity, vascular blurring of the hepatic artery or portal vein and subcutaneous tissue thickness. However, these results are not enough to diagnose mild NAFLD [4]. NAFLD patients have subcutaneous thickness of more than 25.6 ± 56 mm. while non NAFLD has subcutaneous tissue thickness less than 19 ± 52 mm [5].

NAFLD can be defined by clinicians with the following criteria: diffuse echogenicity, uniformly heterogeneous liver, subcutaneous thickness > 2 cm, liver filling the entire field with no edges, attenuation of image quickly within 4–5 cm of depth. With these measurements, the accuracy of ultrasonography in the diagnosis of NAFLD increased by 80% sensitivity and 99% specificity [6].

Fibroblast growth factor 21 (FGF21) is a plasma biomarker which is positively correlated with intrahepatic triglyceride content (IHTC). Elevations of FGF21 are correlated with liver triglycerides, which may progress to end-stage steatosis [7].

Also, plasma levels of FGF21 are correlated with age, dietary interventions, liver weight and body weight. So, FGF21 is an emerging plasma biomarker to diagnose NAFLD [8]. So, we investigated the combined effect of ultrasonography and FGF21 for diagnosis of NAFLD, especially early stages (mild NAFLD).

PATIENTS AND METHODS

This study was conducted at the Internal Medicine Department, Zagazig University Hospitals in collaboration with Medical Biochemistry and Molecular Biology, and Radiology Departments, Faculty of Medicine, Zagazig University. The participants attended the outpatient clinics in the period between July 2019 and July 2022.

The included patients' criteria were the age group from 20 to 75 years old with no sex predilection, overweight and obese subjects who are defined by body mass index (BMI) ≥25 kg/m^2 or waist circumference (WC) ≥85 cm in males or 80 cm in females.

All participants were exposed to the anthropometric measurements, blood pressure measurement, fasting blood glucose (FBG), two-hour post prandial blood glucose (PP2), insulin, glycated hemoglobin (HbA1C), total lipid profile, liver function tests and HOMA IR. Plasma FGF21 was determined using enzyme-linked immune sorbent assay (ELISA).

Received: 22/10/2022
Accepted: 21/12/2022

Abdominal ultrasonography was performed with the following parameters involved in ultrasonographic evaluation: assessment of the size of the liver in both the midline and mid-clavicular lines, the surface of the liver and its echogenicity.

NAFLD was diagnosed by ultrasonography using a high-resolution B-mode ultrasound system according to the guidelines for the diagnosis and management of NAFLD.

Participants who possessed two of the following three characteristics could be diagnosed as fatty liver: the near-field echo of the liver is diffusely increased and more than in the kidney; the intrahepatic duct structure is unclear; the far-field echo of the liver is decreased gradually [9].

Any participants with acute or chronic liver disease, hepato-biliary disease, malignancy, hypothyroid or hyperthyroid disorders were excluded from the study.

Ethical consent:

All participants included in the study gave a written consent and the study gained approval from the IRB of the Faculty of Medicine, Zagazig University (IRB#: 9832-20-9-2022). This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

***Statistical* analysis**

Data collected and encoded using Microsoft Excel software. Data were then imported into Statistical Package for Social Sciences (SPSS version 20.0) software for analysis. Qualitative data were represented as number and percentage, while quantitative data were represented by mean ± standard deviation (SD).

The following tests were used to test the differences for significance: Chi-square tests for difference and association of qualitative variable, t test for quantitative independent groups, and Pearson's correlation for differences between quantitative independent groups. *P* values were set at $\leq$0.05 for significant results and $\leq$0.001 for highly significant results.

RESULTS

NAFLD represented 20.6% of the group under study. Compared to non NAFLD, NAFLD cases were significantly higher regarding BMI, WC, FBG, PP2, HbA1C, HOMA IR, total cholesterol, low-density lipoprotein cholesterol (LDL), triglycerides (TG), aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) (P<0.001 for each). Additionally, high-density lipoprotein cholesterol (HDL) was significantly lower in NAFLD compared to non NAFLD. Moreover, NAFLD cases were significantly associated with male gender (P=0.049), hypertension, diabetes mellitus (DM), obesity, high WC and metabolic syndrome (P<0.001 for each) (**Table 1**).

Serum FGF21 levels were significantly higher among NAFLD cases compared to non NAFLD (190.24±23.94 pg/mL and 134.35±23.08 pg/mL, respectively). Regarding ultrasonography characteristics, intrahepatic triglyceride content (IHTC) was significantly higher among NAFLD cases compared to non NAFLD (13.46±3.21 and 4.41±1.02, respectively) (**Table 1**).

Table (1): Clinical and biochemical characteristics of the studied groups:

Variable			Non-NAFLD (N=270)	NAFLD (N=70)	t/X^2	*P*
Age			49.06±9.36	51.05±8.27	1.856	0.058
Body mass index (kg/m^2)			30.62±6.0	36.64±6.95	7.226	<0.001**
Waist circumference (cm)			101.7±11.51	117.15±13.92	9.564	<0.001**
Systolic blood pressure (mmHg)			129.29±19.15	132.85±17.91	1.756	0.067
Diastolic blood pressure (mmHg)			87.66±11.91	88.8±12.47	1.644	0.087
Fasting blood glucose (mg/dL)			104.95±25.05	154.51±27.55	12.849	<0.001**
Two-hour postprandial (mg/dL)			155.14±36.6	261.62±64.26	12.748	<0.001**
Glycated hemoglobin (gm%)			6.2±1.06	8.24±1.09	14.153	<0.001**
HOMA IR			2.14±0.36	2.67±0.45	10.086	<0.001**
Total cholesterol (mg/dL)			184.51±20.02	228.78±55.65	10.193	<0.001**
High density lipoprotein-cholesterol (mg/dL)			47.84±4.73	41.78±7.40	8.391	<0.001**
Low density lipoprotein- cholesterol (mg/dL)			149.39±15.18	182.94±45.35	10.180	<0.001**
Triglycerides (mg/dL)			126.63±21.51	146.04±35.93	5.559	<0.001**
Aspartate transaminase (U/L)			26.23±5.73	35.8±8.02	6.152	<0.001**
Alanine transaminase (U/L)			25.68±4.24	37.05±8.11	7.330	<0.001**
Alkaline phosphatase (U/L)			57.87±13.02	71.42±16.52	4.849	<0.001**
Fibroblast growth factor 21			134.35±23.08	190.24±23.94	17.914	<0.001**
Intrahepatic triglyceride content			4.41±1.02	13.46±3.21	27.434	<0.001**
Sex	Male	N	154	49	3.88	0.049*
		%	57%	70%		
	Female	N	116	21		
		%	43%	30.		
Hypertension	-ve	N	201	36	13.94	<0.001**
		%	74.4%	51.4%		
	+ve	N	69	34		
		%	25.6%	48.6%		
Diabetes Mellitus (DM)	-ve	N	203	11	84.28	<0.001**
		%	75.2%	15.7%		
	+ve	N	67	59		
		%	24.8%	84.3%		
Obesity	-ve	N	148	17	20.74	<0.001**
		%	54.8%	24.3%		
	+ve	N	122	53		
		%	45.2%	75.7%		
High waist circumference	-ve	N	148	17	20.74	<0.001**
		%	54.8%	24.3%		
	+ve	N	122	53		
		%	45.2%	75.7%		
Dyslipidemia	-ve	N	235	48	13.58	<0.001**
		%	87%	68.6%		
	+ve	N	35	22		
		%	13%	31.4%		
Metabolic syndrome	-ve	N	265	58	27.36	<0.001**
		%	98.1%	82.9%		
	+ve	N	5	12		
		%	1.9%	17.1%		
Total		N	270	70		
		%	100%	100%		

**: highly significant difference; *: significant difference.

Concerning ROC, there were significant areas under curves (AUC) with significant cutoff for FGF-21 and IHTC (>160 and >5.79, respectively, *P*<0.001 for each). The sensitivity was 83.3% for FGF21 and 87.8% for IHTC, and the specificity was 80% for FGF-21 and 85% for IHTC. Interestingly, after combination of FGF21 and IHTC, the sensitivity increased to 88.3% and the specificity was 80% (**Table 2**).

Table (2): Receiver operator curve (ROC) for the studied parameters:

Area Under the Curve						Sensitivity	Specificity
Test Result Variable(s)	Area	Cutoff	P-value	95% Confidence Interval			
				Lower Bound	Upper Bound		
FGF21	0.933	>160	<0.001**	0.904	0.963	83.3%	80%
IHTC	0.968	>5.79	<0.001**	0.950	0.986	87.8%	85%
Combined						88.3%	80%

FGF-21: fibroblast growth factor 21; IHTC: intrahepatic triglyceride content; **: highly significant difference.

FGF 21 was significantly positively correlated with IHTC, age, BMI, WC, FBG, PP2, HA1C, HOMA, cholesterol, LDL, TG, AST, ALT and ALP. However, it was significantly negatively correlated with HDL (*P*<0.001 for each). Regarding IHTC, it was significantly positively correlated with FGF-21, age, BMI, WC, SBP, DBP, FBG, PP2, HA1C, HOMA, cholesterol, LDL, and TG but significantly negatively correlated with HDL (**Table 3**).

Table (3): Correlation between FGF 21 and IHTC and different parameters:

Variable		FGF21	IHTC
Intrahepatic triglyceride content	***r***	0.488	
	P	<0.001**	
Age	***r***	0.226	0.139
	P	<0.001**	0.010*
Body mass index	***r***	0.240	0.279
	P	<0.001**	<0.001**
Waist circumference	***r***	0.348	0.347
	P	<0.001**	<0.001**
Systolic blood pressure	***r***	0.058	0.180
	P	0.289	<0.001**
Diastolic blood pressure	***r***	0.062	0.204
	P	0.255	<0.001**
Fasting blood glucose	***r***	0.346	0.419
	P	<0.001**	<0.001**
Two-hour postprandial blood sugar	***r***	0.322	0.447
	P	<0.001**	<0.001**
Glycated hemoglobin	***r***	0.355	0.536
	P	<0.001**	<0.001**
HOMA IR	***r***	0.290	0.460
	P	<0.001**	<0.001**
Total cholesterol	***r***	0.478	0.260
	P	<0.001**	<0.001**
High density lipoprotein- cholesterol	***r***	-0.437-	-0.191-
	P	<0.001**	<0.001**
Low density lipoprotein- cholesterol	***r***	0.440	0.253
	P	<0.001**	<0.001**
Triglycerides	***r***	0.338	0.125
	P	<0.001**	0.022*
Aspartate transaminase	***r***	0.372	-0.002-
	P	<0.001**	0.973
Alanine transaminase	***r***	0.400	0.072
	P	<0.001**	0.186
Alkaline phosphatase	***r***	0.269	0.016
	P	<0.001**	0.764

**: highly significant difference; *: significant difference.

DM, obesity and metabolic syndrome were significant independent predictors of NAFLD (*P*<0.001, 0.049 and 0.012, respectively) (**Table 4**).

Table (4): Multivariate logistic regression for independent predictors of NAFLD:

Variable	Wald	*P-value*	OR	95% CI	
				Lower	Upper
Sex	2.536	0.111	0.565	0.280	1.141
Hypertension	2.921	0.087	1.866	0.912	3.815
Diabetes Mellitus	41.403	<0.001**	28.595	10.297	79.412
Obesity	3.839	0.049*	8.349	1.122	18.32
Dyslipidemia	3.429	0.064	2.287	0.953	5.491
Metabolic syndrome	6.311	0.012*	7.402	1.553	35.287

**: highly significant difference; *: significant difference; OR: odds ratio; CI: confidence interval

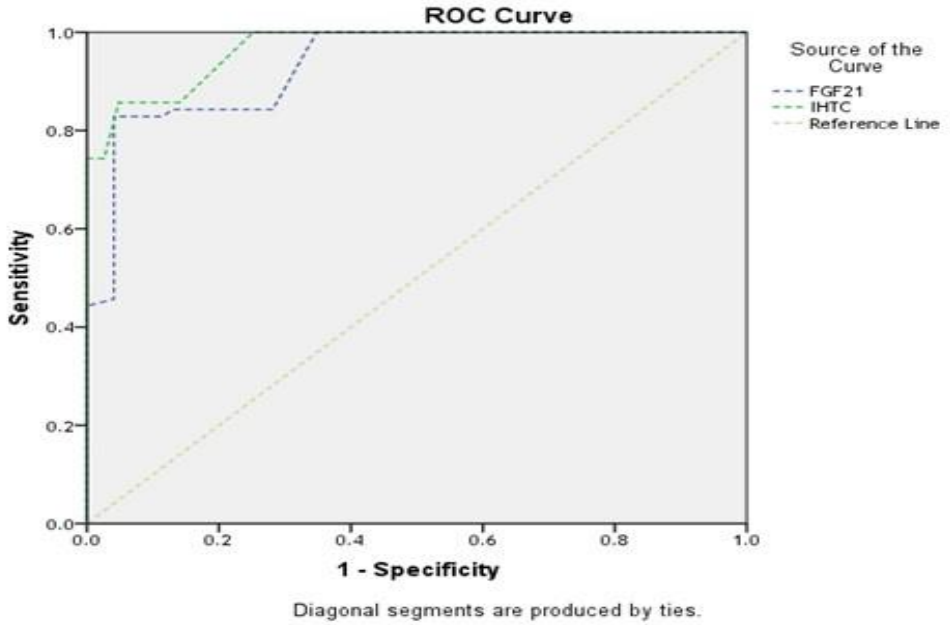

Figure (1): ROC curve for FGF21 and IHTC regarding NAFLD.

DISCUSSION

Nonalcoholic fatty liver disease (NAFLD) is considered now one of the most burden liver disorders as it ranges from simple steatosis to malignant steatohepatitis with cirrhosis and hepatocellular carcinoma (HCC). [1]. NAFLD is diagnosed as hepatic steatosis detected by histology or imaging studies in the absence of 2ry causes of fat accumulation according to the American Association for the Study of Liver Diseases. The best diagnostic method for NAFLD is liver biopsy. Due to biopsy risk and misinterpretation of results, every effort is done to fine new tools to diagnose NAFLD. Bedsides, ultrasound is the best recommended tool for screening and diagnosis of NAFLD as it is easy, noninvasive, cost effective and reliable method. The characteristic ultra-sonographic findings of NAFLD include bright hepatic echos, increased hepatorenal echogenicity, vascular blurring of hepatic artery or portal vein and subcutaneous tissue thickness [9].

The most common limitation of ultrasound is in the accurate diagnosis of mild fatty steatosis (IHTC <30%). So, we decided in our study to use ultrasonography along with FGF21 to increase the ability to diagnose NAFLD and mild fatty steatosis. FGF 21 is a potential plasma biomarker to diagnose NAFLD which is significantly correlated with IHTC [10]. FGF21 is correlated with body weight, liver weight, intrahepatic fat content and increased dramatically with age [11].

In our study, there is a strong correlation between NAFLD and each of BMI and WC. These findings are similar to the results found by **Golabi *et al.*** [12]**.** Also, there is a positive correlation between NAFLD and FBG, HbA1C, HOMA IR and lipid profile. These findings are similar to those found by **Shen *et al.*** [13]. Moreover, our study had revealed that there is a high significance between FGF21 and NAFLD. This finding is similar to that found by **Yan *et al.*** [10]. Regarding ultra-sonographic findings, IHTC was significantly higher among NAFLD cases, which is in agreement with a study done by **Machado *et al.*** [5].

In our study, there is a positive correlation between NAFLD and male gender, hypertension, DM, obesity, ALT, AST, ALP and metabolic syndrome. These results are close to the results in a study done by **Younousi *et al.*** [14]. Our study showed that there is a significant positive correlation between IHTC, ALT, AST, TG,

LDL and total cholesterol, but negative correlation with HDL, which is in agreement with **Fisher *et al.*** [11].

Our study had showed that DM, obesity and metabolic syndrome are predictors of NAFLD irrespective of the other variables.

CONCLUSION

The combined use of ultrasound and FGF21 is better in mild NAFLD diagnosis than using each tool separately. Searching for other parameters in combination with ultra-sonography for better diagnosis, follow up and treatment of NAFLD are recommended.

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