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Original Article

Fibroblast Growth Factor 21 in Obese Children with Metabolic Associated Fatty Liver Disease

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Abstract:
Background: Fibroblast growth factor 21 (FGF21) is an endocrine hormone expressed by the liver. It boosts glucose and lipid metabolism, and insulin sensitivity.
Aim of the work: study the accuracy of FGF21 in diagnosis of metabolic associated fatty liver disease (MAFLD) in obese children.
Subjects and Methods: Children recruited from the Obesity Clinic, Children's Hospital, Ain-Shams University were enrolled in the study. Anthropometric data, biochemical test results; including liver function tests, fasting lipid profile, serum glucose, serum insulin, insulin resistance -assessed by calculating HOMA-IR-, liver ultrasound score for MAFLD, and FGF21 levels were analyzed by ELISA. Patients were subdivided by ultrasound into group A obese children with normal liver and group B obese children with fatty liver.
Results: This study included 39 (60%) males and 26 (40%) females, with mean ± SD age of 10.17 ± 2.54 years. Their body mass index (BMI) standard deviation score (SDS) was 3.36 ± 0.61, the mean waist/hip ratio (W/H ratio) was 0.93 ± 0.12, mean± SD systolic blood pressure was 1.36 ± 1.47 and diastolic blood pressure was 1.76 ± 0.84. 32 (49%) cases had insulin resistance (mean HOMA-IR = 3.81 ± 3.01), 21(32%) fulfilled the criteria of metabolic syndrome and 27 (41.5%) had MAFLD. Group A included 38 (58.8%) cases, and group B included 27 (41.5%) cases. Patients in group B had statistically higher SDS of BMI, SDS of waist circumference, SDS of hip circumference (p-value= < 0.001, p < 0.001, and p < 0.001 respectively). In group A 12 (31.5%) had dyslipidemia, 1(2.6%) had elevated TG and low HDL, 2 (5.3%) had elevated cholesterol and elevated LDL, 7 (18.4%) had elevated LDL and 2 (5.3%) had low HDL, while in group B, 22 (81.4%) had dyslipidemia, 10 (37%) had elevated TG, 16 had low HDL, 20 (74%) had increased LDL and 7(25%) had increased cholesterol (p=0.001). Mean FGF21 was 169.08 ± 153.68 pg/dl after 12 hours fasting (normal value= up to 115 pg/dl in children. Mean ± SD FGF21 in group A was 96.05 pg/dl ± 39.70 with median 90 pg/dl, while mean FGF21 in group B was 271.85 pg/dl ± 192.69 SD with median 180 pg/dl (p=0.001). FGF21 cutoff value of 115 pg/dl was diagnostic of fatty liver with sensitivity of 88.9%, specificity of 73.7% (p=0.001). FGF-21 correlated positively with US finding (p=0.001).
Conclusion: Obese children with MAFLD had higher levels of FGF21. FGF21 value of 115 pg/dl may be added as a non-invasive biomarker for the diagnosis of MAFLD in obese children.

Keywords: Children; obesity; fibroblast growth factor 21; metabolic associated fatty liver disease
Abbreviations: BMI: Body mass index; ELISA: Enzyme linked immunosorbent assay; FGF21: Fibroblast Growth Factor 21; HOMA-IR: Homeostasis model of assessment of insulin resistance; IR: insulin resistance; MAFLD: Metabolic associated fatty liver disease; SD: standard deviation; SDS: standard deviation score

Introduction

Metabolic dysfunction-associated fatty liver disease (MAFLD) formerly known as non-alcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease in childhood (*1*)**.** It progresses to cardiometabolic involvement; the metabolic dysfunction-associated steatotic liver disease (MASLD). Due to the growing number of obese children, the prevalence of fatty liver increased. For many years, MASLD has been perceived as a hepatic consequence of insulin resistance (IR). However, fatty liver can precede type 2 diabetes and metabolic syndrome and may even be a risk factor for their development (*2*, *3*)**.** MASLD may progress from steatosis to the more severe metabolic associated steato-hepatitis (MASH), (formerly known as non-

alcoholic steatohepatitis (NASH)), fibrosis, and cirrhosis which may end in liver failure or hepatocellular carcinoma. Although, liver biopsy and histology and the gold standard for differentiating MASLD from normal liver, however, it is an invasive maneuver and impractical for screening purposes. MASLD is defined by the histologic evidence of at least 5% of the hepatic steatosis in the absence of other causes of excessive liver fat accumulation, it ranges from simple hepatic MAFLD, steatosis to steatohepatitis; a more serious stage of MASLD, with or without fibrosis, to possibly cirrhosis and end stage liver disease (*4*)**.**

Obesity and features of the metabolic syndrome have been closely linked to MASLD development. Due to the increased prevalence of obesity worldwide, MASLD has reached epidemic proportions over time. In fact, recent data reported a mean prevalence of MASLD ranging from 7.6% in general population up to 34.2% in obese children (*5*, *6*)**.** Given its unfavorable cardiometabolic burden and impact on renal function, pediatric MASLD represents a worrying phenomenon needing a more comprehensive and successful management (*7*)**.** Non-invasive surrogate markers of MASLD include anthropometric measurements (e.g., body mass index (BMI), waist circumference, waist/hip ratio, biochemical data including hyperlipidemia, hypertriglyceridemia, homeostasis model of assessment values for insulin resistance [HOMA-IR], adipokine patterns (e.g., leptin and adiponectin) in MASLD patients, has led to the search for a biomarker that could be associated with the occurrence of MASLD in obese children (*6*). Fibroblast growth factor 21 (FGF-21) is an endocrine hormone mainly expressed by the liver, adipose tissue, skeletal muscle, and pancreas. It boosts glucose and lipid metabolism, and insulin sensitivity. Some studies reported that FGF-21 has a protective effect against hepatic steatosis and liver damage (*2*)**,** however, other studies reported conflicting results; thus, the role of FGF-21 in MASLD needs further investigation. Increased serum FGF-21 levels were also reported in patients with type 2 diabetes mellitus (T2DM) and may indicate a role in pathogenesis of T2DM. Elevated levels also correlate with fat content in nonalcoholic fatty liver disease and positively correlate with BMI suggesting obesity as FGF-21 resistant state (*2*). The aim of this study was to study the accuracy of FGF21 in diagnosis of MAFLD in obese children.

Subjects and Methods

This cross-sectional study was conducted at Obesity Clinic, Children's Hospital, Ain Shams University, Egypt. All participants were enrolled after verbal informed consent was obtained from parents or guardians. The study was approved by the Research Ethics Committee of Ain Shams University Hospitals and according to the guidelines of Institutional Review Board (IRB) of Faculty of Medicine with approval number of FMASU MS 330/2020.

Participants

The study included on 65 obese children with BMI > 97th percentile of normal for sex and age. Children with viral hepatitis, autoimmune hepatitis, chronic liver disease, any child receiving steroids, or other obesity inducing drugs or children with associated comorbidities like cardiac or renal comorbidities were excluded. Children with syndromic obesity as Prader- Willi syndrome, and Bardet-Biedl syndrome were also excluded. Parents and guardians received verbal information about the study design and objectives, and they all gave a verbal consent to participate in the study. Participation was voluntary and they were ensured that they could discontinue participation at any time.

Methods

Detailed history was collected: including dietetic history, history of comorbidities as diabetes mellitus, hypertension, any chronic disease, obesity inducing medications.

Anthropometric measurements were recorded in the morning after at least 8 hours fasting. Participants were lightly dressed, barefoot during measurement of weight and height. Weight was measured in kg (to the nearest 100 grams) using an electronic digital scale and its accuracy was periodically verified using reference weights. Height was measured in cm (measured to the nearest mm), children were measured using standing Harpendens stadiometer (Holtain limited, UK), with the subject standing with his back against the gauges and feet on the weighing platforms. Weight for height was plotted on growth curves and SDS of weight for height was calculated. Height and weight percentiles were checked according to World Health Organization (WHO) growth charts (*8*), and were plotted on growth curves. BMI was calculated and SDS values for BMI were used according to national BMI reference (*9*). Waist circumference (WC) was measured midway between lowest rib and the iliac crest to the nearest 0.1 cm at the end of normal expiration. Hip circumference (HC) was measured at the point of maximal protrusion of

the buttocks, with the tape parallel to the floor. The measurement was taken at the end of normal expiration (*10*). The waist to hip ratios (W:H ratio) were calculated.
Abdominal ultrasound was performed with the patient supine. Liver span was obtained in the mid clavicular line. Both were obtained while the probe was oriented longitudinally. Measures were applied to the pediatric chart to assess the presence of hepatomegaly according to the age and height of the child. The examination was done using Philips EPIQ5, (Philips IU Elite, USA), with an abdominal 5 to 12 MHz probe transducer. This abdominal ultrasound machine was used to screen for MAFLD. Based on the results, participants were categorized into cases (those with MAFLD) and control group (those without MAFLD). Liver steatosis and severity was determined in each participant using ultrasonography by an expert radiologist. Liver steatosis severity was assessed non-invasively -without liver biopsy-. It relied on ultrasonography using three scoring items. The Hamaguchi score relied on sonographic findings (*11*) and comprised: (1) Bright liver and hepatorenal echo contrast (score 0-3). Score 0: both liver echogenicity and hepatorenal contrast was normal; score 1: either liver echogenicity or hepatorenal contrast was increased; score 2: both liver echogenicity and hepatorenal contrast were mildly increased; score 3: both liver echogenicity and hepatorenal contrast were significantly increased. (2) Deep attenuation of the diaphragm (score 0-2). Score 0: the diaphragm could be clearly distinguished by an observer; Score 1: visualization of the diaphragm was obscure, but an observer could distinguish the diaphragm; Score 2: an observer could not distinguish the diaphragm. (3) Visualization of intrahepatic vessels (score 0-1). Score 0; no evidence of vessel blurring; Score 1: the border of intrahepatic vessels was unclear and the lumen of intrahepatic vessels were narrowed.

Patients were subdivided into group A obese children with normal liver by ultrasound (control) and group B obese children with fatty liver. Group A was designated for those with score 0, where patients had homogenous (normal) echo pattern and liver was average in size for age. Group B comprised patients with score 2 with bright homogenous (fatty) liver and liver was mildly enlarged for age.(*11*) MAFLD was considered the hepatic manifestation of metabolic syndrome (3 of 5 are diagnostic= abdominal obesity, insulin resistance (IR), high serum triglycerides, low serum high-density lipoprotein and hypertension) (*12*).

The following tests were performed: liver function tests (alanine aminotransferase (ALT), aspartate aminotransferase (AST), lipid profile (Total cholesterol, triglycerides, high density lipoproteins (HDL-C), low density lipoproteins (LDL-C), fasting serum glucose (but not blood sugar and glycated hemoglobin) and fasting serum insulin. The remaining serum was stored at -20 degree Celsius till the time of use for assessment of FGF-21. Analysis of chemistry analytes was performed on AU 680 chemistry analyzer according to manufacturer instructions (Beckman Instruments Inc., Scientific Instruments Division, Inc., CA92634-3100, USA). LDL-C concentration was calculated through the Friedewald equation [total cholesterol – (HDL-C + triglycerides/5)] (*13*).

The reference values in our study were calculated according to age: cholesterol was considered high if ≥ 200 mg/dL, LDL-C ≥ 100 mg/dL, HDL < 40 mg/dL and triglycerides were considered if ≥ 150 mg/dL. AST and ALT reference values were up to 40 IU/L. Albumin reference interval was 3.8- 5.4 mg/dL.

Analysis of insulin was performed by an electrochemiluminescence immunoassay on the fully automated analyzer Cobas e 411 (Roche Diagnostics, Indianapolis, USA). Insulin resistance was assessed by calculating HOMA-IR using the formula: (fasting glucose x fasting insulin)/405, Normal HOMA-IR was 0.5-1.4, ≥1.9 indicates early insulin resistance, and ≥ 2.9 shows insulin resistance. (*14*). The FGF-21 concentrations were determined using a double-antibody sandwich enzyme-linked immunosorbent assay kit (ELISA) (Code E1983Hu) (Bioassay laboratory technology, Zhejiang, China). Samples were collected after 12 hours fasting. Serum of non-hemolysed samples were stored at – 20 Celsius till analysis of FGF-21. Repeated freezing and thawing were avoided.

Statistical Analysis

Student t-test and Mann-Whitney test: were used to assess the difference between the two groups in numerical data for parametric and non-parametric data, respectively. Chi-square test: was used for comparison of categorial data. Spearman/Pearson correlation test: was used to correlate between serum FGF-21 levels and other numerical parameters. Receiver operating curve (ROC) analysis: was used for evaluation of the diagnostic accuracy of serum levels of FGF-21 in the diagnosis of fatty liver. Odds ratio: was used as a measure of association between an exposure and an outcome. The OR represented the odds that an outcome will occur with a given particular exposure, compared to the odds of outcome in the absence of that exposure. Regression analysis: (Binary logistic regression analysis) was used for prediction of fatty liver and linear

regression analysis for prediction of FGF-21 levels. The result was considered significant if p-value < 0.05. Sample size was estimated by Department of Community Medicine, Faculty of Medicine, Ain Shams University using the G* power program version 3.1 (Universität Düsseldorf, Germany). The data were coded, and analyzed by the Statistical Package for the Social Sciences software program version 23.0 (SPSS Inc., Chicago, Illinois, USA).

Results

This study included 65 obese children; their ages ranged from 6.1 to 15.3 years with a mean ±SD of 10.2 ± 2.5 years. They were 39 males (60%) and 26 females (40%). Their baseline sociodemographic characteristics, anthropometric measurement and blood pressure of are shown in Table 1. Systolic hypertension was encountered among 45 (69.2%) cases and 53 (81.5%) had diastolic hypertension. Abdominal examination of the studied cases showed mild hepatomegaly with firm consistency, rounded edge and smooth surface evident in 15 cases and 30 cases (46%) had acanthosis nigricans. Ultrasound assessment of the studied patients showed that 27 (41.5%) patients had mild hepatomegaly (41.5%) and 27 (41.5%) had bright homogenous liver. None of the studied children had hepatic focal lesions or dilated intrahepatic biliary radicles. (Table 1).

Table 1. Baseline characteristics, anthropometric measurements and blood pressure of the studied cohort of obese children

	All cohort		Group A Number= 38		Group B Number= 27		P value
	Number	%	Number	%	Number	%	
Sex							
Males	39	60	21	55.3	18	66.7	0.355
Females	26	40	17	44.7	9	33.3	0.355
	Mean ±SD	Range	Mean ±SD	Range	Mean ±SD	Range	
Age (years)	10.2 ± 2.5		9.15 ± 2.18		11.61 ± 2.34		0.001
Weight for height (SDS)	11.65 ± 5.02		13.02 ± 5.87		13.02 ± 5.87		0.083
Height for age (SDS)	1.03 ± 1.56		1.13 ± 1.60		0.9 ± 1.53		0.444
BMI	31.72 ± 5.64		28.29 ± 2.72		36.56 ± 5.11		< 0.001
BMI SDS	3.36 ± 0.61		3.13 ± 0.59		3.68 ± 0.5		< 0.001
Waist circumference (SDS)	4.5 ± 1.85		3.84 ± 1.63		6.05 ± 2.59		< 0.001
Hip circumference (SDS)	4.2 ±1.85		3.14 ± 1.82		5.32 ±1.92		< 0.001
Waist/Hip ratio	0.93 ± 0.211		0.94 ± 0.15		0.91 ± 0.08		0.931
SDS SBP	1.56 (0.5-3.5)		1.39 ± 0.62		1.31 ± 2.18		0.337
SDS DBP	1.76 ± 0.84		1.72 ± 0.80		1.81 ± 0.91		0.724
	Number	%	Number	%	Number	%	
SBP elevated	45		29	64.4	16	35.6	0.23
SBP normal	20		9	45	11	55	
DBP elevated	53		34	64.2	19	35.8	0.051
DBP normal	12		4	33.3	8	66.7	
U/S Liver imaging	Number	%	Number	%	Number	%	
-Liver mildly enlarged	27	41.5	0	0	27	41.5	< 0.001
-Liver average size	38	58.5	38	58.5	0	0	< 0.001
-Homogenous (normal)	38	58.5	38	58.5	0	0	< 0.001
-Bright homogenous (fatty)	27	41.5	0	0	27	41.5	< 0.001
-No focal lesions	65	100	38	58.5	27	41.5	0.355
-No dilated biliary vessels	65	100	38	58.5	27	41.5	0.355
-B-right liver score (0-3)			Score 0	38(100%)	Score 1	4(14.5%)	
					Score 1-2	23(86.1%)	
					Score 2	3(11.1%)	
-Deep attenuation of diaphragm score (0-2)			Score 0	38(100%)	Score 1	24(88.9%)	
					Score 2	3(11.1%)	
-Visualization of intrahepatic vessels (0-1)			Score 0	38(100%)	Score 1	2(7.4%)	

BMI: Body mass index; DBP: Diastolic blood pressure; SBP: Systolic blood pressure; SDS: Standard deviation score

The obese group without fatty; liver group A (n=38) and obese group with fatty liver; group B (n=27) were different. Those in group B were statistically older in age (p<0.001), were statistically more obese for age (p<0.001), taller (p=0.002), had more SDS of BMI (p<0.001), more SDS of waist circumference (p<0.001), and more SDS hip circumference (p<0.001). No statistical difference was found in the W:H ratio (p=0.93), SDS of SBP (p=0.337) or the SDS of DBP (p=0.724) between the 2 groups. The mean cholesterol of the cohort was161.72 ± 33.23mg/dL,

(median=152, range= 102- 245mg/dL). While the mean cholesterol in group A was 154.21 ± 29.92 245mg/dL, (median= 150.5, range=150.5 – 124.29mg/dL) and in group B the mean cholesterol was 172 ± 35.3mg/dL (median= 176, =137 – 207.6mg/dL) (p= 0.077). (Table 2).

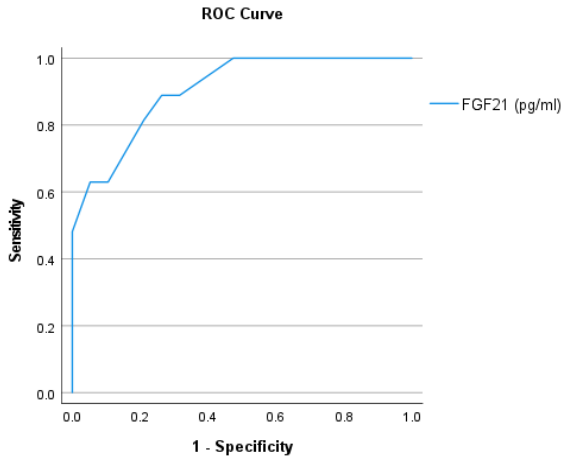
In the current study, there were 45 (69.2%) had insulin resistance of them 40 (88.8%) cases with dyslipidemia, 9(22.5%) cases had hypercholesterolemia, 11(27.5%) cases with hypertriglyceridemia, 27 (57.5%) cases showed increased LDL-C and 19 cases with decreased HDL-C. Furthermore, isolated cases for hypercholesterolemia, hypertriglyceridemia, increased LDL-C and decreased HDL-C have been found in 4(10%), 9(22.5%), 4 (10%) and 7(17.5%) cases respectively. Combined dyslipidemia was found in 13 (65%) cases out of the 65 cases, and 21 cases fulfilled the criteria of metabolic syndrome. They had central (abdominal)obesity (by increased waist/hip ratio) and at least 2 other risk factors as elevated blood pressure according to age, high fasting blood glucose and presence of low HDL or hypertriglyceridemia.

Insulin resistance was detected by (HOMA-IR ≥ 1.9) in 32 (49.2%) cases, while 19 (29.2%) cases showed early insulin resistance (HOMA-IR ≥ 2.9). Mean FGF21 was 169.08 ± 153.68 pg/dl, median value (IQR) was 120 (70-180) pg/dL. FGF21 was normal (< 115 pg/ml) in 31 cases, and it was elevated (>115pg/ml) in 34 cases. The comparison between FGF-21 levels, lipid profile and HOMA-IR in group A (normal liver) and group B (fatty liver) are shown in Table 2. Statistical difference was found between the 2 groups regarding FGF-21, triglycerides, LDL-C, AST and ALT. (Table 2).

Table 2. Statistical comparison between FGF-21 levels, Lipid profile and HOMA-IR in the studied cohort

	Obese Group A (n=38) No fatty liver by ultrasound		Obese Group B (n=27) With Fatty Liver by ultrasound		t /z score	P value
	Mean ± SD	Median (IQR)	Mean ± SD	Median (IQR)		
FGF-21 (pg/mL)	96.05 ± 39.7	90.00	271.85 ± 192	180	0.99	< 0.001
Cholesterol (mg/dL)	154.2 ± 29.9	150.5	172 ± 35.3	176	2.22	0.03
Triglycerides (mg/dL)	102.9 ± 40.5	87.5	156.19 ±56.7	170	4.4	< 0.001
LDL-C (mg/dL)	95.6 ± 22.55	92	110.9 ± 27.5	115	2.4	0.02
HDL-C (mg/dL)	42.55 ± 8.04	42.5	42.88 ± 11.4	40	0.42	0.67
AST (IU/L)	27.63 ± 17.9	35.44	35.44 ± 14.1	38	1.1	0.005
ALT (IU/L)	26.64 ± 15.2	20.5	35.3 ± 15.34	38	1.7	0.001
HOMA-IR	3.73 ± 2.08	2.4	3.86 ± 3.55	3.30	1.6	0.213

ALT: alanine aminotransferase; AST: aspartate aminotransferase; FGF2: fibroblast growth factor 21; TG: triglycerides; LDL: low density lipoprotein; HDL: high density lipoprotein; HOMA-IR: Homeostasis model assessment of insulin resistance



Sensitivity %	88.9
Specificity %	73.7
Confidence interval	95%
P value	< 0.001

Figure 1. ROC analysis of FGF-21 levels Diagnostic Accuracy of MAFLD
FGF-21: Fibroblast Growth Factor-21, ROC curve: Receiver Operating Characteristic Curve

Waist circumference correlated positively with SBP (r= 0.43, p < 0.001), but not with DBP (r = 0.167, p= 0.18). FGF-21 showed positive correlation with older age (p= 0.016), weight for height (p=0.001), BMI (p< 0.001), SDS of BMI (p < 0.001), SDS waist circumference (p= 0.001), SDS of

waist circumference (p= 0.001), SDS hip circumference (p= 0.002), triglycerides (p < 0.001), and ALT (p < 0.001), but not with height (p = 0.23), W:H ratio (p= 0.72), SDS SBP (p= 0.37) and SDS DBP (p= 0.27).

Table 3. ROC analysis of FGF-21 and BMI levels diagnostic accuracy of MAFLD

	AUC	Cutoff	Lower Bound	Upper Bound	Sensitivity %	Specificity %	P value
FGF21	0.905	115 pg/mL	0.837	0.974	88.9	73.7	< 0.001
BMI	0.786	30	0.84	0.732	89	70	0.001

AUC: Area under curve; BMI: body mass index; FGF-21: Fibroblast Growth Factor-21

ROC analysis revealed that FGF-21 cutoff value of 115 pg/dL detected cases of fatty liver with an area under the curve (AUC) of 90.5%, sensitivity of 88.9%, specificity of 73.7% and p-value of < 0.001. (Table 3 and Figure 1). Binary logistic regression analysis revealed that the BMI and FGF-21 levels were statistically significant predictors of fatty liver by ultrasound. (Table 4). Linear regression analysis demonstrated that triglycerides level, weight for height, SDS of hip circumference and SDS of BMI were predictors of FGF-21 levels. (Table 5).

Table 4. Binary Logistic regression analysis of FGF-21 and BMI for diagnostic accuracy of MAFLD

		Confidence interval		OR	P value
		Lower Bound	Upper Bound		
MAFLD	FGF-21	1.002	1.056	1.671	0.036
	BMI	1.172	2.383	1.028	0.005
	Waist Circumference	1.012	1.052	1.21	0.045
	Systolic Hypertension	1.045	1.087	1.13	0.366
	HOMA IR	1.006	1.057	1.07	0.351

BMI: body mass index; FGF-21: Fibroblast Growth Factor-21; HOMA IR: Homeostasis model of assessment of insulin resistance

Table 5. Linear regression analysis of predictors of FGF-21 levels among the studied cohort

		Confidence interval 95%		t	P value
		Lower Bound	Upper Bound		
FGF-21	Triglycerides (mg/dL)	0.664	1.549	5.007	< 0.001
	LDL-C (mg/dL)	1.014	2.692	4.419	< 0.001
	Weight (SDS)	3.692	7.287	6.113	< 0.001
	Height (SDS)	-3.759	-0.374	-2.444	0.018
	Waist circumference (SDS)	2.21	8.64	5.012	0.001
	Hip circumference (SDS)	-6.866	-2.895	-4.92	0.001
	BMI SDS	2.21	8.64	3.196	0.002

BMI: body mass index; FGF-21: Fibroblast Growth Factor-21; LDL= Low density lipoproteins; SDS= standard deviation score.

Discussion

Our study provides evidence that FGF-21 has moderate sensitivity and specificity in non-invasive diagnosis of MAFLD in obese children. Fasting FGF-21 was diagnostic of MAFLD among obese children in our studied cohort (p < 0.001). The sensitivity and specificity of fasting FGF-21 in diagnosis of MAFLD was moderate (88.9% and 73.7%). This moderate sensitivity and specificity does not allow FGF-21 to stand alone as a non-invasive surrogate diagnostic marker for diagnosis of MAFLD. Hence, it cannot replace the golden standard diagnosis by liver biopsy, but it may be included within a scoring system to improve sensitivity and specificity of other non-invasive diagnostic scores, i.e. the NAFLD fibrosis score, etc. The fasting FGF-21 has a very short life span and is typically affected by the immediate previous meal carbohydrate-content. Maybe FGF-21 is not a suitable diagnostic marker, but it may prove valuable for follow up purposes.

The propensity of systemic systolic hypertension among our studied cohort is disturbing (69.2%). Our study provides alarming concerns about the necessity of regular blood pressure assessment among obese children. We did not study the echocardiographic findings among these children, and we are not aware if this systolic hypertension was associated with cardiac

hypertrophy or not, as it was out of the scope of our study. More prospective long-term studies into the precipitating factors for this systolic hypertension, its management and complications are needed to fill the gap of knowledge. It seems that other factors are involved in the pathogenesis of this hypertension and not simply obesity. It may be related to more sodium content or life style choices (*15*). FGF-21 is reported to be protective in MAFLD, and is reported to mitigate the hypertension as well (*16*). We did not study the diets of our studied cohort as it was out of the scope of our study.

Lipid profile and weight measures were predictors of FGF-21 levels in our studied cohort (p=0.001). Again, it is not clear if the FGF-21 levels represent the protective cascade to dyslipidemia. But in any case, dyslipidemia and weight measurements are readily available tests crucial for diagnosis of MAFLD.

Waist circumference and waist/hip ratio together with FGF-21 correlated with the sonographic findings of MAFLD and metabolic syndrome in our cases (p=0.001). The BMI and BMI SDS associated with waist circumference measurements remain a golden standard for diagnosis of obesity. As BMI alone has limitations; of them that it cannot differentiate muscle from fat (*17*). BMI SDS in our study was found to be predictive of FGF-21 among our studied cohort. The FGF-21 seems to have other protective roles in childhood obesity; hence its level may reflect the mitigated physiologic response to obesity than an obesity associated factor.

Limitations of our study were multiple. As we did not compare the sonographic findings with the liver biopsy findings, we could not delineate the FGF-21 among those with MAFLD and those with MASLD. We relied on the sonographic findings but not the fibroscan or controlled attenuation parameter (CAP), hence we did not study the correlation of FGF-21 to these parameters which are thought to have higher non-invasive accuracy in diagnosis of MAFLD and MASLD. CAP is reported to have 83% sensitivity and 100% specificity (*18*), yet, combing CAP with FGF-21 sensitivity and specificity remain to be studied.

Conclusion

Waist circumference and waist/hip ratio together with FGF-21 correlated with the sonographic findings of MAFLD and metabolic syndrome in our studied children with obesity. Lipid profile and weight measures were predictors of FGF-21 levels. FGF-21 has moderate sensitivity and specificity as a diagnostic marker for MAFLD or MASLD and maybe incorporated in future scoring system for diagnosis or follow up parameter of outcome of MAFLD or MASLD. Children with obesity have very high propensity of hypertension that warrants investigation and possible management.

Author Contributions

All authors contributed to the study's conception and design. All authors prepared the materials. AA supervised the whole work. PS collected the data. DK and NA analyzed the data and wrote the first draft of the manuscript, and all authors commented on previous versions. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest in connection with the reported study.

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