The Turkish Journal of Pediatrics 2022; 64: 671-682
https://doi.org/10.24953/turkjped.2021.1876

Original Article

Identifying the effects of excess weight, metabolic syndrome and insulin resistance on liver stiffness using ultrasound elastography in children

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ABSTRACT

Background. Metabolic syndrome (MetS) and insulin resistance (IR) are known predictors of nonalcoholic fatty liver disease (NAFLD) which is one of the significant comorbidities of obesity. Obese children with MetS and IR are reported to be more likely to have advanced liver fibrosis compared to those without MetS or IR. The aim of this study is to determine the effects of excess weight, MetS and IR on liver fibrosis assessing liver stiffness in children using ultrasound elastography and compare gray scale ultrasonographic findings of hepatic steatosis (HS) with liver fibrosis.

Methods. The study group involved 131 overweight/obese children. The control group involved 50 healthy lean children. Groups were adjusted according to body mass index (BMI) and BMI-standard deviation scores (SDS). Liver stiffness measurements which are expressed by shear wave velocity (SWV) were performed for each individual. The study group was further subgrouped as children with MetS and without MetS, with IR and without IR.

Results. The mean SWV of liver was 1,07 ± 0,12 m/s in the control group and 1,15 ± 0,51 m/s in the study group. The difference was significant (p=0,047). SWV of liver was weakly correlated with age, BMI, BMI-SDS, Homeostatic Model Assessment-Insulin Resistance and high-density lipoprotein cholesterol. The mean SWV of the liver in the study group for children without MetS was 1,1 ± 0,44 m/s, with MetS was 1,23 ± 0,70 m/s. The difference was not significant (p=0,719). The mean SWV of the liver in the study group for children without IR was 1,02 ± 0,29 m/s, with IR was 1,24 ± 0,61 m/s. The difference was not significant (p=0,101). In multivariate regression analysis, the only independent factor affecting liver stiffness was BMI-SDS (OR:2,584, 95% CI: 1,255-5,318, p=0,010).

Conclusions. Obesity itself, regardless of MetS or IR seems to be the major problem affecting liver stiffness in this study. However, large scale longitudinal studies might clarify this issue.

Key words: childhood obesity, metabolic syndrome, insulin resistance, shear wave elastography, liver stiffness.

Childhood obesity is a significant health problem in industrialized countries.[1] The prevalence rates for obesity and being overweight were found at 9.8% and 23.2%, respectively in the Turkish population.[2] The prevalence of metabolic syndrome (MetS) and insulin resistance (IR) is increasing in parallel with the rise of the proportion of the obese population. The prevalences are at 33% and 43% for MetS and IR, respectively in obese Turkish children.[3,4] Abnormal glucose metabolism, dyslipidemia, and hypertension are the main features of MetS.[5]

The increase of nonalcoholic fatty liver disease (NAFLD) in obese children is worrisome.[6] NAFLD is a general name of a

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Received 22nd June 2021, revised 1st September 2021, 6th December 2021, accepted 5th April 2022.

spectrum of diseases varying from hepatic steatosis to nonalcoholic steatohepatitis (NASH). Accumulation of fat in hepatocytes progressively can cause inflammation and fibrosis, which is called NASH.[7] NASH may cause cirrhosis, malignancy, and organ failure in children. Also, after liver transplantation, survival is shorter in NASH compared with the general population. Children with NASH, have increased liver-related mortality compared with children of the same age in the general population.[8-12] Obese children with MetS and IR are reported to be more likely to have advanced liver fibrosis compared to those without MetS or IR.[13,14]

Liver biopsy is the trademark for the assessment and classification of NAFLD. However, it has some risks associated with its invasiveness. Technical difficulties, anesthesia requirements, small tissue sample size, and nonsuitability for patient follow-up are limitations of this procedure.[15] Thus, a non-invasive imaging modality is needed to assess liver fibrosis. Conventional Ultrasound (US) is the preferred noninvasive imaging modality for assessing liver steatosis with its safety, low cost, and easy access. However, detecting mild steatosis or differentiating steatosis from fibrosis are the limitations of this modality.[16] Lately, ultrasound based elastography has emerged as non-invasive imaging modality in the assessment of liver tissue stiffness. The working principle of elastography is based on the tissue stiffness. There are two types of ultrasound-based elastography techniques; static and dynamic. Strain elastography (SE) is the static method. Shearwave Elastography (SWE), Acoustic Radiation Force Impulse Elastography (ARFI) and Transient elastography (TE) are the dynamic types. SWE is an elastography technique that forms in vivo shear waves in the interested organ by generating transient tissue deformation using US forces. The square root of tissue elasticity is proportional to shear wave velocity (SWV).[17,18] After initiating the pulse, the measurement is displayed either in m/s or kPa.[19] Under pathological conditions such as fibrosis, as parenchymal tissue gets stiffer, the shear wave velocity (SWV) increases.[7] In point (p)-SWE, using real-time B-mode ultrasound imaging, an ROI is placed on the parenchymal tissue to perform the measurements. Real-time ultrasound imaging identifies the large vessels and masses which is avoided in the parenchymal measurements.[19] SWE is a promising technique for the non-invasive staging of liver fibrosis in children.[20-25]

The SWV measurements (m/s) of liver were classified in three categories in the literature; the values <1.20 m/s regarded as normal, ≥1.20 m/s -<1.60 m/s regarded as insignificant fibrosis, ≥ 1.60 m/s regarded as significant fibrosis.[6,18]

In the present work, our goals were to assess the effects of obesity on liver stiffness and to compare the liver stiffness of overweight/obese children with MetS and IR with those without MetS and IR using p-SWE, and also to compare gray scale ultrasonographic findings of hepatic steatosis (HS) with fibrosis categories.

Material and Methods

This study was approved by the Erciyes University, Medical School, Ethics Committee (approved number 07.03.2018-134). Signed informed consent was obtained from the children's parents according to the World Medical Association Declaration of Helsinki, revised in 2000, Edinburgh.

Study population

Children included in the study were between the ages of 6 and 18 years. One hundred thirty-one overweight/obese children were included in the study group, and 50 lean children were included in the control group. The participitants of the study and control groups were mainly adjusted according to the body mass index (BMI), BMI- standard deviation scores (SDS).

Almost all of the overweight/obese children included in the study were referred from the Division of Pediatric Endocrinology or

Division of Pediatric Nutrition and Metabolism. Medical and laboratory histories were carefully investigated. Children with chronic inflammatory or autoimmune diseases, acute or chronic viral hepatitis, and using drugs known to cause steatosis were not included in the study group.

The control group was formed by lean children without any signs of liver disease. The majority of them had visited the pediatric radiology division with urinary incontinence, urinary infection, nephrolithiasis or chronic abdominal pain. Medical histories and laboratory findings were investigated for the presence of any systemic diseases before sonographic examination. Children with any abnormality in liver echo structure on ultrasonography were excluded from the control group.

Anthropometric and clinical characteristics

At the onset, BMI of all individuals was obtained. BMI is calculated by dividing weight by the square of the height. In addition to BMI, BMI-SDS were calculated according to growth charts using age and sex.[26] Individuals with more than 1 SDS above the median were grouped into the study group. Individuals with 1 SDS above the median to 2 SDS below the median were grouped as the control. Systolic and diastolic blood pressure of all individuals were measured. Laboratory tests for all individuals included were as follows: fasting blood glucose, fasting insulin, triglycerides, high-density lipoprotein (HDL) cholesterol, aspartate aminotransferase (AST), and alanine aminotransferase (ALT). The time interval between laboratory measurements and p-SWE measurements was around 0-5 days.

Individuals were evaluated for MetS and IR. International Diabetes Federation (IDF) criteria were used to evaluate MetS.[27] The MetS diagnosis was established if the patient had altered abdominal circumference and two or more of the following criteria; fasting blood glucose ≥100 mg/dl, triglycerides ≥ 150 mg/dl, HDL≤ 40 mg/dl, taking a lipid-lowering drug, systolic blood pressure ≥ 130 mmHg, diastolic blood pressure ≥85 mmHg, and taking an antihypertensive drug. There are no established cut–off values for glucose metabolism, dyslipidemia, and arterial hypertension for children below 10 years. Pelin et al.[28] defining pediatric MS, used adapted IDF criteria. They added the criteria, TG ≥ 95th percentile, and BP ≥ 95th percentile for age and sex, in addition to the above criteria. Therefore, children younger than 10 years old, fulfilling the above criteria (only 3 children) were classified in MetS, in this study. Homeostatic Model Assessment-Insulin Resistance (HOMA-IR) levels were used to evaluate IR. The following formula is used for calculating HOMA-IR: fasting plasma glucose (mg/dL) × fasting plasma insulin (IU/mL)/405.[29] HOMA-IR is a good indicator of insulin resistance. As the value gets higher, the severity of insulin resistance gets higher. Cut-off values for HOMA-IR were regarded as 2.67 in boys and 2.22 in girls for the prepubertal period and 3,16 in both genders for the pubertal period.[30,31]

The study group was divided into subgroups as children with MetS and without MetS, with IR and without IR.

US Measurements

Ultrasound examinations were performed with Siemens Acuson S3000 using a 6C1 transducer. P-SWE was used for elastography measurements. SWV is measured in an ROI. A single radiologist with an experience of 15 years in abdominal ultrasonography and two years in SWE, who was blinded to the children's laboratory findings performed the examinations.

Patients were laid in supine position for the examination. Gray scale ultrasonography was performed to examine the liver echotexture. Hepatic steatosis (HS) was scored as grade 0, 1, 2, 3 according to liver echotexture, clarity of blood vessels and distinguishability of the diaphragm and liver parenchyma in echo amplitude.[32] SWE measurements were obtained

during the patient's free breathing, using intercostal approach, from the liver's right lobe, approximately from the same location for all individuals. Measurements were obtained at a depth of 4-5 cm from the skin. The ROI was placed about 3 cm beneath the liver capsule in an area of homogeneous parenchyma, free of visible vessels. Ten valid measurements were performed for all individuals, and the mean value is recorded (Figs. 1 and 2). The entire exam duration time took approximately 10 minutes.

The SWV measurements (m/s) of liver ≥ 1.20 m/s were regarded as meaningful for fibrosis.[6,18]

Statistical Analysis

Statistical analysis was conducted with SPSS IBM Statistics Version 22.0. Values were expressed as the mean ± SD and range (minimum to maximum). The Shapiro Wilk test was used to confirm the normality of distribution for continuous variables. The Student's t-test or Mann-Whitney U test was used to compare continuous variables between two groups according to the normality of distribution. Pearson correlation was used for correlation analysis. To compare the categoric variables the χ^2 test was used. To determine the variables affecting liver stiffness, univariate

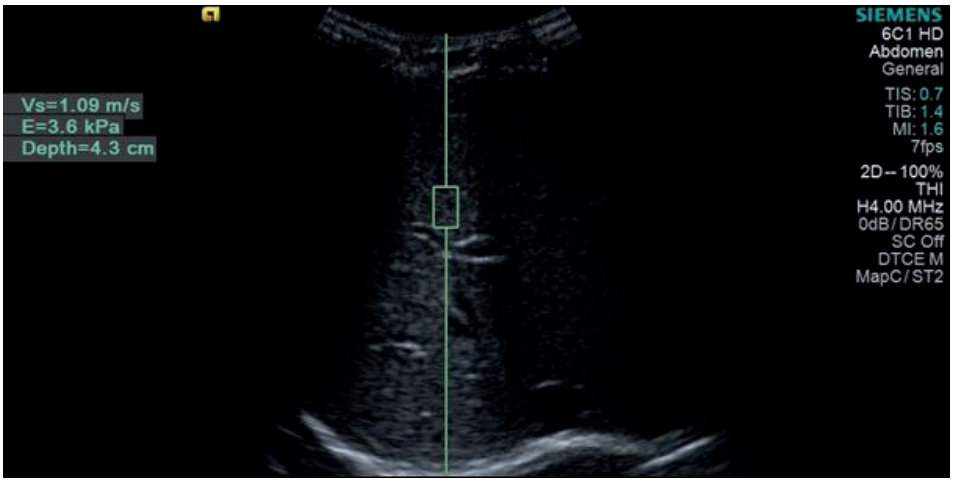

Fig. 1. SWE measurement of an 11 year old girl in control group.

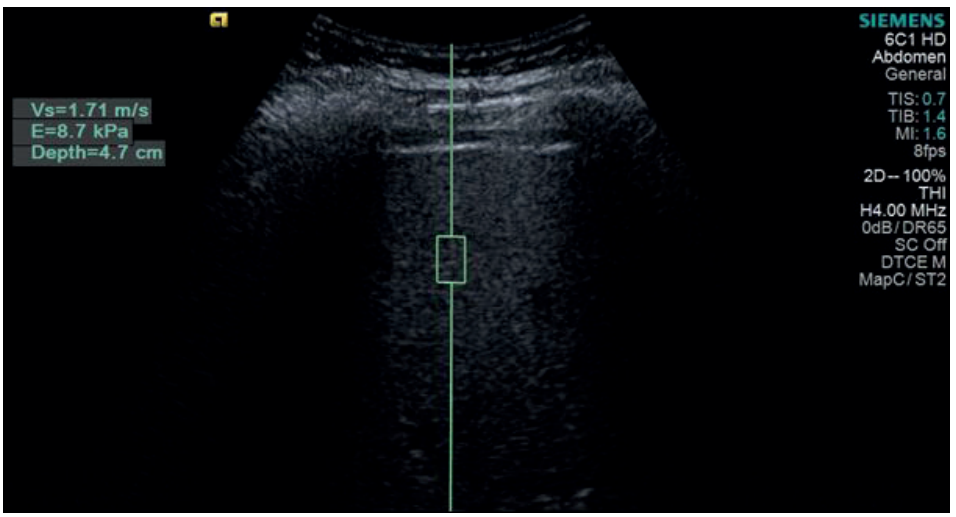

Fig. 2. SWE measurement of a 14 year old boy in study group.

logistic regression analysis was performed. Multivariate logistic regression analysis was performed to identify the independent factor for liver stiffness after adjusting for age. Interclass correlation coefficient was used for reliability measurements of p-SWE. Differences were regarded as significant at $p<0.05$.

Results

Demographic, anthropometric, metabolic, and laboratory parameters of the study and the control group are compared in Table I.

No statistically significant differences were found between the study and control group for

Table I. Comparison of demographic, anthropometric, metabolic and laboratory parameters of the study and control group.

	Control group (n=50)	Study group (n=131)	
	mean ± SD (range)	mean ± SD (range)	*p* value
Males (n (%))	14 (28%)	58 (44.3%)	0.067
Age (years)	12.5 ± 3.5 (6.2-18.9)	13 ± 2.7 (6.4-18)	0.92
Weight (kg)	42.7 ± 15.3 (15-83)	70.3 ± 20.4 (25.3-124.3)	<0.001
Height (cm)	149.1 ± 18.5 (104-183.5)	153.7 ± 14.2 (117.3-188)	0.166
BMI (kg/m2)	18.6 ± 2.9 (13.9-26.2)	29.1 ± 4.6 (18.3-42.8)	<0.001
BMI-SDS	-0.26 ± 0.84 (-1.69- 1.00)	2.4 ±0.6 (1.1-4.2)	<0.001
SBP(mmHg)	101 ±7.7 (90-120)	110.8 ± 14.4 (90-150)	0.04
DBP (mmHg)	63.1 ± 9.5 (50-80)	69.3 ± 8.8 (50-90)	0.013
Fasting blood glucose(mg/dl)	86.8 ± 9.4 (61-107)	86.9 ± 7.8 (65-121)	0.905
Fasting insulin (microıu/ml)	10 ± 7.8 (4.4- 15.8)	18.8 ± 12.3 (1.4-76.4)	0.021
Triglycerides (mg/dl)	83.8 ± 33.9 (28-147)	128.1 ± 75.9 (41-524)	0.004
HDL cholesterol (mg/dl)	55.6 ± 10.5 (40-80)	45.4 ± 10.4 (28.4-86)	<0.001
AST (IU/L)	23.1 ± 5.6 (15-40)	26.1 ± 13 (10-100)	0.173
ALT (IU/L)	14.9 ± 4.8 (9-31)	25.5 ± 20.4 (6-163)	<0.001
HOMA-IR	3.2 ± 3.5 (0.91-12.4)	4.10± 2.9 (0.30-17)	0.101
SWV of liver (m/s)	1.07 ± 0.12 (0.78-1.35)	1.15 ± 0.51 (0.63- 3.36)	0.047

BMI: body mass index, BMI-SDS: body mass index-standard deviation score, SBP: systolic blood pressure, DBP: diastolic blood pressure, HDL cholesterol: high-density lipoprotein cholesterol, AST: aspartate aminotransferase, ALT: alanine aminotransferase, HOMA-IR: homeostatic model assessment-insulin resistance, SWV: Shear wave velocity

gender, age, height, and blood glucose. In the study group, the mean SWV was 1.20 ± 0.56 m/s in girls and 1.08 ± 0.43 m/s in boys. The difference was not significant (p = 0.18). In control subjects, the mean SWV was 1.07 ± 0.13 m/s in girls and 1.07 ± 0.11 m/s in boys. The difference was not significant (p = 0.91). The mean SWV of the study group was significantly higher than the control group. The mean SWV was 1.07 ± 0.12 m/s and 1.15 ± 0.51 m/s for control and study group, respectively (p= 0.047).

Correlation analysis was performed between SWV and anthropometric, metabolic parameters for all individuals (Table II). SWV showed a weak positive correlation with age, BMI, BMI-SDS and HOMA-IR and a weak negative correlation with HDL.

The relation between hepatic steatosis (HS) and fibrosis categories was analyzed with χ^2 test Although no statistically significant difference was found between HS and fibrosis categories ($\chi 2$ =2.423, p=0.65), 15 overweight-obese children with no or mild steatosis had SWV values over 1,60 m/s.

All of the individuals were evaluated for criterias of MetS and IR. None of the subjects in the control group met any of the criteria for MetS or IR. Twenty-nine out of 131 overweight-obese children were diagnosed with MetS. Seventy-five of them was diagnosed with IR. The study group was subgrouped as with MetS, without MetS and with IR, without IR. The relationship between subgroups of MetS and IR was also analyzed using χ^2 test. A statistically significant difference was found between these subgroups. (χ^2 =5.271, p=0.022) (Table III).

While 41% of overweight-obese children who were not grouped in MetS had IR, 5% of overweight-obese children who did not have IR was grouped in MetS. Therefore, subsequent analyses were performed for MetS and IR subgroups, separately.

SWV measurements, anthropometric, metabolic, and laboratory parameters were compared in subgroups, with MetS and without MetS (Table IV). No statistically significant difference was found between the two subgroups for gender and age, height and diastolic blood pressure, blood glucose, AST, and ALT. Statistically significant differences were found for most of the MetS parameters as expected. The mean SWV was 1.1 ± 0.44 (m/s) and 1.23 ± 0.70 (m/s) for the subjects without MetS and with MetS, respectively. The difference was not significant (p=0.719).

Table II. The results of correlation analysis between SWV and anthropometric, metabolic parameters.

	Shear-wave velocity (m/s)
Age	0.154
	0.038
BMI	0.319
	<0.001
BMI-SDS	0.185
	0.018
Fasting glucose	-0.085
	0.278
AST	-0.007
	0.932
ALT	0.051
	0.525
HOMA-IR	0.199
	0.020
Triglycerides	0.020
	0.808
HDL cholesterol	-0.202
	0.014

The first line is r value, the second line is p value for all parameters. BMI: body mass index, BMI-SD: body mass index-standard deviation, HDL cholesterol: high density lipoprotein cholesterol, HOMA-IR: homeostatic model assessment-insulin resistance, AST: aspartate aminotransferase, ALT: alanine aminotransferase, SWV: Shear wave velocity

Table III. The relationship between MetS and IR.

	Without IR	With IR
Without MetS	37.4 %	40.5 %
With MetS	5.3 %	16.8 %

(χ^2 =5.271, p=0.022)

SWV measurements, anthropometric, metabolic, and laboratory parameters were compared in subgroups, without IR and with IR (Table IV). No statistically significant difference was found for age. Anthropometric, metabolic and laboratory parameters were statistically different between these subgroups except for systolic, diastolic blood pressure, AST, and ALT. The mean SWV was 1.02±0.29 m/s and 1.24±0.6 m/s for overweight-/obese children without IR and with IR, respectively. The difference was not significant (p=0.101).

Univariate regression analysis was used for analyzing the relationship between the parameters and liver fibrosis (SWV≥1.20). Age, gender, BMI-SDS, MetS and IR were chosen as independent variables. Multivariate regression analysis was used for assessing the parameters that are independently associated with liver fibrosis after adjusting for age. The only significant independent predictor of liver stiffness was BMI-SDS (OR: 2.584, %95 CI:1.255-5.318, *p*=0.010) (Table V).

Table IV. Comparison of SWV measurements, anthropometric, metabolic and laboratory parameters were compared in study group between children without MetS and with MetS, without IR and with IR.

	Without MetS	With MetS	*p*	Without IR	With IR	*p*
	N=102	N=29		N=56	N=75	
	mean ± SD (range)	mean ± SD (range)		mean ± SD (range)	mean ± SD (range)	
Males (%)	44(43.1 %)	14 (48.3%)	0.62	34(60.7 %)	24(32.0 %)	<0.001
Age (years)	12.3±2.7 (6.4-18)	13.4±2.6 (7-17.2)	0.069	12.1±2.9 (6.4-18)	12.9±2.4 (6.9-17.2)	0.70
BMI (kg/m^2)	28.4±4.5 (18.3-42.8)	31.4±4.4 (23.8-40)	0.002	27.3 ± 4.5 (18.3-38.8)	30.4 ± 4.3 (23.7-42.8)	<0.001
SDS	2.4±0.6 (1.1-4.2)	2.7±0.6 (1.2-3.8)	0.029	2.2±0.6 (1.1-3.8)	2.6 ± 0.6 (1.2-4.2)	0.001
Systolic blood pressure (mmHg)	108.2±12.6 (90-140)	117.8±16.7 (90-140)	0.023	101±7.7 (90-140)	112.1±13.9 (90-150)	0.323
Diastolic blood pressure (mmHg)	68.3±7.1 (50-80)	71.8±12.1 (50-90)	0.364	67.9±8.9 (50-85)	70.1 ± 8.7 (60-90)	0.462
Triglycerides (mg/dl)	107.3±53.4 (41-367)	200.8±96.5 (63-524)	<0.001	112.7±67.7 (41-391)	139.4±80.1 (41-524)	0.008
HDL cholesterol (mg/dl)	47.8±10.1 (28.4-86)	37.3±6.7 (30-58)	<0.001	48.4±11.4 (30-86)	43.3 ± 9.2 (28.4-71)	0.004
AST (IU/L)	25.46±11.90 (10-100)	28.40±16.31 (13-88)	0.625	27.3±13.9 (10-100)	25.2±12.3 (11-88)	0.207
ALT (IU/L)	24 ±16.3 (6-95)	31.5±31.6 (10-163)	0.212	24.3±17.6 (6-88)	26.5±2.5 (9-163)	0.338
HOMA-IR	3.7±2.4 (0.3-12.4)	5.6±3.9 (0.9-17)	0.012	1.8±0.8 (0.30-3.12)	5.7±2.8 (2.81-16.98)	<0.001
SWV of liver (m/sn)	1.1±0.44 (0.70-3.18)	1.23±0.70 (0.63-3.36)	0.719	1.02±0.29 (0.63-2.14)	1.24 ± 0.61 (0.63-3.36)	0.101

BMI: body mass index, BMI-SDS: body mass index-standard deviation, SBP: systolic blood pressure, DBP: diastolic blood pressure, HDL cholesterol: high-density lipoprotein cholesterol, AST: aspartate aminotransferase, ALT: alanine aminotransferase, HOMA-IR: homeostatic model assessment-insulin resistance, SWV: Shear wave velocity, MetS: metabolic syndrome, IR: insulin resistance

Table V. The results of univariate and multivariate regression analysis assessing the parameters that are independently associated with liver fibrosis.

	Liver stiffness (SWV ≥1.20m/sn)					
	Univariate analysis			Multivariate analysis adjusted for age		
Parameters	OR	%95 CI	*p* value	OR	%95 CI	*p* value
Age	1.203	1.055-1.371	0.006			
Gender	0.579	0.272-1.1230	0.155			
BMI-SDS	1.321	0.954-1.830	0.094	2.584	1.255-5.318	0.010
MetS	0.967	0.368-2.540	0.946			
IR	0.304	0.120-0.768	0.012			

BMI-SDS: body mass index- standard devaiation score, MetS: metabolic syndrome, IR: insulin resistance

The intraobserver agreement which is expressed as interclass correlation coefficient was 0.83 (95 % CI, 0.79-0.87; p <0.001). The results demonstrated that the SWV measurements had good agreement reproducibility.

Discussion

This study was mainly interested in the effects of excess weight and metabolic parameters on liver stiffness using SWE in children. In the first step, the relation between excess weight and liver stiffness was assessed. Although p value (0.047) was close to 0.05, the mean SWV of the overweight/obese children was statistically higher than the control group. With more control and study subjects the difference might be more obvious. In a study by Bailey et al.[33], SWV measurements were significantly lower in the normal group than the obese group concordant with the current study. The mean SWV was 1.08±0.14 m/s and 1.44±0.39 m/s in order of the healthy and obese group. The value for healthy group was in close agreement with the current findings. However, the value for the obese group was relatively higher than the results of the current study, indicating more liver stiffness. This difference may be related to their study population consisting mostly of Hispanic children who have a predisposition for obesity-based abnormalities, such as liver diseases and diabetes.[34] On the other hand, in a study using TE.[35] no significant difference was found for liver stiffness measurements between overweight, obese, and healthy children. Berná-Serna et al.[6] observed a weak positive correlation between SWV and BMI. In concordance with their research, there was a weak positive correlation between SWV and BMI, BMI-SDS in the present study. Age was positively associated with SWV. This result contributed to the studies, stating that liver stiffness measurements have an age-dependent increase.[33,36]

In the second step, qualitative assessments of conventional US was compared with p-SWE measurements. No statistically significant difference was found between fibrosis categories and HS categories. Bailey et al.[33] reported that SWV is significantly higher in abnormally hyperechoic livers than livers with normal echoes on conventional US. The present results were compatible with their study. Berná-Serna et al.[6] found significant differences between fibrosis categories and HS grades. Nine obese or overweight children out of 148 with normal liver echotexture or mild steatosis on the grayscale showed significant fibrosis in SWE measurements of their study. Unlike Berná-Serna et al.[6] the difference between fibrosis categories and HS grades in the current study was not significant. However, it was intriguing that 15 overweight or obese children out of 116 with normal echotexture or mild steatosis, showed significant fibrosis on SWE measurements, similar with their study.

Although MetS and IR are closely related metabolic factors, they are actually different entities.[37] Kurtoglu et al.[37] reported that IR was

prominent in obese patients not only with MetS but also without MetS. Also, they stated that IDF criteria of MetS were not sufficient to discover patients with IR. Based on this study, the effects of IR are analyzed separately in this study. Contributing to their study, 41% of overweight-obese children who were not grouped in MetS had IR, 5% of overweight-obese children who did not have IR were grouped in MetS.

The association of liver stiffness and MetS, IR was studied in the following steps. No statistically significant difference was found between the mean SWV of the patients with MetS and without MetS. Also, there was no statistically significant difference for SWV between the patients with IR and without IR. Due to the coexistence of visceral obesity, IR, dyslipidemia, NAFLD is considered to be the hepatic manifestation of MetS.[38,39] Some studies in adults state that MetS is associated with a higher liver fibrosis degree in subjects with NAFLD.[40-42] A study assessing the effect of MetS on liver stiffness in children using TE stated that, fibrosis is three times more likely to occur in the presence of the MetS.[13] In the current study, although the patients with MetS have higher SWV measurements than patients without MetS, the difference was not statistically significant. The association of IR and liver stiffness was reported in some studies.[43-47] A few pediatric studies[46,47] were encountered in the literature, determining the effects of IR on liver stiffness. Kwon et al.[46] utilizing TE, Stepanov et al.[47] using SWE, reported that liver stiffness measurements were correlated with HOMA-IR. In the current study, SWV measurements were weakly correlated with HOMA-IR (p=0,020). However, there was no significant difference for SWV between patients with or without IR. On the other hand, in a higher age group, the effects of these factors on liver stiffness may be more evident as the duration of MetS and IR increase.

In the last step of the study, regression analysis was performed for determining the most significant factors affecting liver stiffness. The only independent factor affecting liver stiffness was BMI-SDS after adjusting for age in the multivariate analysis. Huh et al.[48], in a large study cohort in adults, categorized their subjects into four groups according to metabolic health status and obesity: metabolically unhealthy obese, metabolically healthy obese, metabolically healthy non-obese, metabolically unhealthy non-obese. They reported that obese patients were at a higher risk for liver fibrosis than non-obese patients regardless of metabolic parameters. Obesity and metabolic abnormalities are regarded as the two underlying mechanisms of NAFLD. A commonly accepted hypothesis on NAFLD pathogenesis is the 'two hits thesis'. Obesity is the 'first hit' increasing the sensitivity of the liver to injury. Metabolic abnormalities are the 'second hit' injuring the liver by oxidative and inflammatory cytokines leading to liver fibrosis.[49-51] In the current study, multivariate analysis revealed that liver stiffness was 2,6 times more likely to occur with the increase of BMI-SDS per one unit (OR:2.584, 95% CI: 1.255-5.318, p=0.010). Similar with Huh et al.[48], the results of the current study point out that, obesity itself has a direct impact on liver fibrosis and join to the formation of liver fibrosis without the 'second hit' step. Obesity was the only significant factor affecting liver stiffness. On the other hand, the duration of MetS and IR was short in this study. In advancing ages with longer duration of these factors, the results would be more accurate.

One of the important limitations of this study was the absence of liver biopsy for histologic confirmation. However, liver biopsy is an invasive, impractical procedure that is not preferred especially in children. The lack of other reference standards such as Magnetic Resonance Elastography is another limitation of the study. This study was a cross-sectional study and is not adequate to see cause and effect relationship. Longitudinal follow-up studies are needed to reveal the factors causing liver fibrosis, accurately.

To our knowledge, the current study is the first to analyze and compare the effects of excess weight, MetS and IR on liver stiffness in children utilizing p-SWE technique.

According to the results of this study obesity itself, rather than MetS or IR, seems to be the major problem affecting liver stiffness. However further, large scale longitudinal studies following children in advancing ages might clarify this issue.

Ethical approval

This study was approved by the Erciyes University Medical School, Ethics Committee (approved number 07.03.2018-134).

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: ZFK, FK, MK, AC; data collection: ZFK, SS, GD; analysis and interpretation of results: ZFK, NH; draft manuscript preparation: ZFK, NH. All authors reviewed the results and approved the final version of the manuscript.

Source of funding

The authors declare the study received no funding.

Conflict of interest

The authors declare that there is no conflict of interest.

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