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Usefulness of Transient Elastography for Non-Invasive Diagnosis of Liver Fibrosis in Pediatric Non-Alcoholic Steatohepatitis

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ABSTRACT

Background: Transient elastography (FibroScan®) is a non-invasive and rapid method for assessing liver fibrosis. While the feasibility and usefulness of FibroScan® have been proven in adults, few studies have focused on pediatric populations. We aimed to determine the feasibility and usefulness of FibroScan® in Korean children.

Methods: FibroScan® examinations were performed in 106 children (age, 5–15 years) who visited the Konyang University Hospital between June and September 2018. Liver steatosis was measured in terms of the controlled attenuation parameter (CAP), while hepatic fibrosis was evaluated in terms of the liver stiffness measurement (LSM). Children were stratified into obese and non-obese controls, according to body mass index (≥ or < 95th percentile, respectively).

Results: The obese group was characterized by significantly higher levels of aspartate aminotransferase (AST, 57.00 ± 48.47 vs. 26.40 ± 11.80 IU/L; $P < 0.001$) and alanine aminotransferase (ALT, 91.27 ± 97.67 vs. 16.28 ± 9.78 IU/L; $P < 0.001$), frequency of hypertension and abdominal obesity (abdominal circumference > 95% percentile) ($P < 0.001$), CAP (244.4–340.98 dB/m), and LSM (3.85–7.77 kPa) ($P < 0.001$). On FibroScan®, 30 of 59 obese children had fibrosis (LSM > 5.5 kPa), whereas the remaining 29 did not (LSM < 5.5 kPa). Obese children with fibrosis had higher levels of AST (73.57 ± 56.00 vs. 39.86 ± 31.93 IU/L; $P = 0.009$), ALT (132.47 ± 113.88 vs. 48.66 ± 51.29 IU/L; $P = 0.001$), and gamma-glutamyl transferase (106.67 ± 69.31 vs. 28.80 ± 24.26 IU/L; $P = 0.042$) compared to obese children without fibrosis. LSM had high and significant correlation ($P < 0.05$) with AST, ALT, homeostasis model assessment for insulin resistance, and AST-to-platelet ratio index.

Conclusion: FibroScan® is clinically feasible and facilitates non-invasive, rapid, reproducible, and reliable detection of hepatic steatosis and liver fibrosis in the Korean pediatric population.

Keywords: Liver Fibrosis; Nonalcoholic Fatty Liver Disease; Non-Invasive Diagnosis; Child

INTRODUCTION

The increasing prevalence of childhood obesity is reflected in the increased prevalence of metabolic syndrome and non-alcoholic fatty liver disease (NAFLD) associated with

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obesity.[1,2] Because liver histology tests require invasive investigations with substantial risk and discomfort for the patient, it is difficult to determine the exact prevalence of NAFLD in children, but it is estimated that NAFLD is a widespread disease, affecting up to one-third of all children worldwide.[3] In particular, NAFLD is reported in 10%–80% of children who undergo liver function tests, and in 15%–44% of children who undergo liver ultrasonography.[4,5] Among adults with NAFLD, 25% have non-alcoholic steatohepatitis (NASH) and many (34%–44%) progress to liver disease within a short time.[6,7] The prognosis of NASH varies widely depending on histological findings. If left untreated, childhood NAFLD can also progress to cirrhosis, leading to severe outcomes. Therefore, it is clinically important to screen obese children for steatohepatitis and fibrosis.[8]

To date, liver biopsy remains the gold standard for detecting liver inflammation and fibrosis in childhood NAFLD. However, liver biopsy has many limitations, including high cost, invasiveness, risk of complications, risk of operator error, and the fact that the small harvested sample of liver tissue may not be representative of the overall state of the liver.[9] These limitations are more pronounced in children, who are at a higher risk of complications. Both the patient's family and the treating physician may be less inclined to consider liver biopsy, which would delay adequate diagnosis and initiation of adequate treatment. For these reasons, there have been ongoing efforts to replace liver biopsy with medical imaging and biochemical testing approaches as effective, non-invasive alternatives for estimating the histological severity of NAFLD.

Transient elastography (FibroScan®), which employs pulse-echo ultrasound to measure the hardness of the liver, has shown excellent accuracy for evaluating fibrosis in adult patients with chronic hepatitis B or hepatitis C, as well as in liver transplant recipients. Although its accuracy in NAFLD is quite lower, FibroScan® is still considered a useful test.[10-13] However, while the usefulness of FibroScan® has been fairly well studied in adults, few research investigations have focused on children, and there is a pronounced shortage of data from Korea.

Therefore, in this study, we aimed to examine the feasibility and usefulness of FibroScan® in children, as well as to investigate the features of the FibroScan®-based liver profile in obese and non-obese children. In particular, we aimed to examine the reliability and validity of FibroScan® by analyzing the correlation between FibroScan®-based parameters and the results of biochemical tests such as liver function parameters that reflect liver fibrosis and the extent of hepatic steatosis.

METHODS

Participants

The study enrolled 106 children aged 5–15 years who visited the pediatric department at Konyang University Hospital as inpatients or outpatients between June 2018 and September 2018 and who successfully underwent FibroScan®. Of the 106 children who were assessed for obesity, 59 had a body mass index (BMI) above the 95th percentile for the same age and sex group; such children were considered obese. Of these, 30 children were confirmed to have liver fibrosis on FibroScan® (liver stiffness measurement [LSM] ≥ 5.5 kPa). For the control group, 47 non-obese children hospitalized for non-liver-related diseases were intentionally selected from the same age and sex groups as those of the obese children (**Fig. 1**).
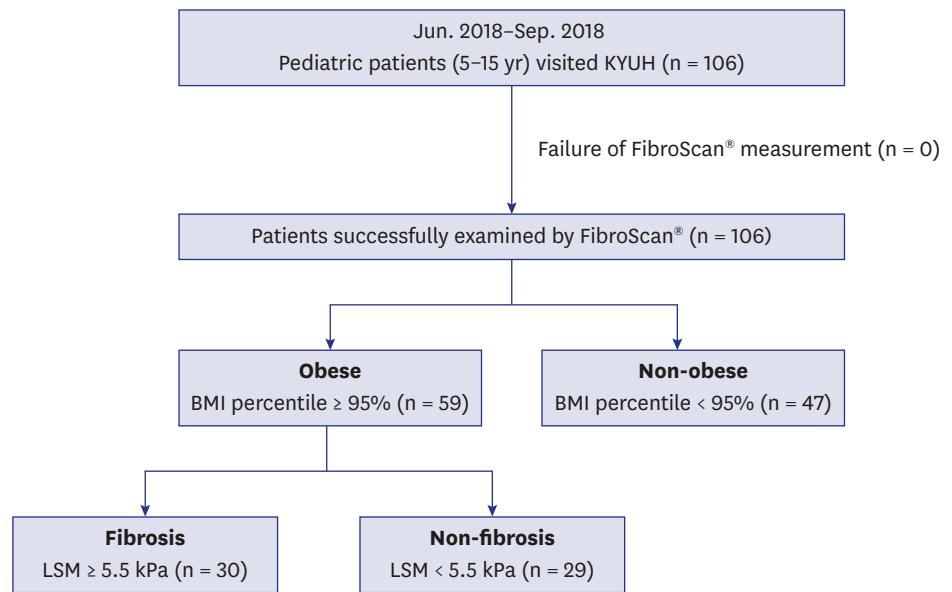
Fig. 1. Flow chart of the study protocol and overview of the study population.
KYUH = Konyang University Hospital, BMI = body mass index, LSM = liver stiffness measure.

Evaluation of liver fibrosis and steatosis

LSM, which is an indicator of liver fibrosis, and the controlled attenuation parameter (CAP), which is an indicator of fat accumulation in the liver, were measured using the FibroScan® device (Echosens, Paris, France) according to the manufacturer's guidelines.[14] All children were evaluated using a standard 3.5-MHz M probe (diameter, 7 mm), which can be used to measure LSM and CAP simultaneously. The FibroScan® S probe (5 MHz; diameter, 5 mm) is designed for young children aged ≤ 2 years who have narrow intercostal spaces, but it can only be used for measuring LSM.[15] Meanwhile, the XL probe (2.5 MHz; diameter, 10 mm) is useful in children with very thick adipose tissue, but it is less accurate than the other probes. Because our study focused on the importance of accurately measuring LSM and CAP in children in the clinical setting, we used the M probe for all children. The test procedure was as follows: with the participant in the prone position, the examiner placed the probe against the skin between the ribs, facing vertically toward the right lobe of the liver. Once the probe is in the correct position, LSM data were displayed in kPa, together with an indication as to whether or not the test was valid. CAP data were displayed simultaneously in dB/m. Once LSM and CAP could be measured successfully 10 times in a row, the early, less precise values were deleted, and the mean of the last 10 valid measurements was calculated and retained. To reduce inter-examiner variance, one skilled examiner conducted the FibroScan® assessment, and only results with an interquartile range below 30% were considered valid. Each test took, on average, approximately 5 minutes.

Clinical and biochemical parameters

Height, weight, BMI, blood pressure (BP), heart rate (HR), and waist circumference were measured on the same day as that of the FibroScan® examination. As biochemical variables, serum platelet counts and the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), triglycerides, and total cholesterol were measured no more than 1 month before the FibroScan® examination. During sample collection, obese groups were all on fasting status, whereas the non-obese groups were mostly on non-fasting status.

The NAFLD fibrosis score, AST-to-platelet ratio index (APRI), and BARD (BMI + AST/ALT ratio + diabetes mellitus) score were evaluated. The NAFLD fibrosis score can take various values from negative to positive, where more positive values are predictive of more severe liver fibrosis. The NAFLD fibrosis score is a composite of age, BMI, diabetes status, AST/ALT ratio, platelet count, and albumin levels. A larger APRI, which is given by higher AST levels and/or lower platelet count, is predictive of more severe liver fibrosis or cirrhosis. The BARD score, which is a composite of obesity status (BMI), AST/ALT ratio, and diabetes status, is scored on a scale of 0–4 points, with scores of ≥ 2 being predictive of more severe liver fibrosis.

Statistical analysis

To compare the obese and non-obese groups, as well as the fibrosis and non-fibrosis subgroups of obese children, we used two-sided t-tests for continuous variables and χ^2 tests or Fisher exact tests for categorical variables. Pearson and Spearman correlation analyses were used to examine the correlations between continuous variables. Relationships with $P < 0.05$ were considered significant in all inferential tests.

Ethics statement

The present study protocol was reviewed and approved by the Institutional Review Board (IRB) of Konyang University College of Medicine (approval No. 2017-11-012). Signed consent forms were obtained from all participants and their parents. The written consent form included information about the study objectives and about possible adverse effects and discomfort associated with the examination. There were separate consent forms for the children and for their guardians. Before the study, consent was obtained from both the child and at least one parent who could communicate in Korean. The IRB approved this consent procedure.

RESULTS

Demographic and clinical characteristics

Of the 106 participants examined, 67 were male, and they were age 10.56 ± 2.62 years. No significant differences in sex or age distribution were noted between the obese group (59 children; 39 male, 66.1%; age, 131.36 ± 29.17 months) and the non-obese control group (47 children; 28 male, 59.6%; age, 120.83 ± 33.38 months).

Compared to non-obese children, obese children had significantly higher weight, height, BMI, AST levels (57.00 ± 48.47 vs. 26.40 ± 11.80 IU/L; $P < 0.001$), and ALT levels (91.27 ± 97.67 vs. 16.28 ± 9.78 IU/L; $P < 0.001$). In addition, the obese group had a higher percentage of hypertensive participants (systolic BP ≥ 95th percentile or diastolic BP ≥ 95th percentile across children of the same sex and age; 35.6%, 21 children vs. 0 children; $P < 0.001$) and participants with a waist circumference ≥ 95th percentile (66.1%, 39 patients vs. 0 children; $P < 0.001$) (**Table 1**).

FibroScan® results

Both CAP, which reflects the intensity of liver steatosis, and LSM, which reflects the severity of liver fibrosis, were significantly higher among obese participants than among non-obese participants (CAP, 292.69 ± 48.29 vs. 204.15 ± 44.25 dB/m; $P < 0.001$ and LSM, 5.81 ± 1.96 vs. 4.47 ± 0.95 dB/m; $P < 0.001$) (**Table 2**). Upon examining the results in detail, CAP showed similar distribution plot in the obese group and the control group, while LSM showed larger distribution in the obese group (**Fig. 2**). Moreover, there was a positive and significant correlation between LSM and CAP, with a correlation coefficient of 0.522 ($P < 0.001$) (**Fig. 3**).

Table 1. Characteristics of the study population

Characteristics	Obese (n = 59)	Non-obese (n = 47)	P value[a]
Sex, male:female	39:23	28:19	0.489
Age, mon	131.36 ± 29.17	120.83 ± 33.38	0.092
Height, cm	150.28 ± 15.85	139.05 ± 18.18	0.001
Weight, kg	62.06 ± 18.54	37.27 ± 15.09	< 0.001
BMI, kg/m²	26.78 ± 3.87	18.46 ± 3.18	< 0.001
Waist circumference, cm	84.41 ± 13.25	57.90 ± 9.12	< 0.001
Systolic BP, mmHg	111.53 ± 16.62	99.21 ± 7.32	< 0.001
Diastolic BP, mmHg	71.81 ± 12.33	63.77 ± 6.57	< 0.001
Heart rate, beats/min	84.15 ± 10.32	77.66 ± 10.71	0.002
Hypertension[b]	21 (35.6)	0 (0)	< 0.001
Waist circumference, ≥ 95th percentile	39 (66.1)	0 (0)	< 0.001
WBC count, cells/µL	8,652.27 ± 2,639.46	8,521.28 ± 3,459.77	0.839
Hemoglobin, g/dL	13.67 ± 1.02	13.24 ± 1.22	0.072
Hematocrit, %	40.35 ± 2.85	39.90 ± 3.67	0.516
Platelets, ×10³ cells/µL	320.55 ± 46.65	281.74 ± 93.79	0.015
CRP, mg/dL	0.73 ± 1.47	1.03 ± 1.48	0.349
Glucose, mg/dL	100.49 ± 12.76	108.00 ± 19.12	0.025
Diabetes mellitus	4 (6.8)	0 (0)	0.069
Total cholesterol, mg/dL	173.97 ± 37.23	146.65 ± 59.07	0.009
AST, IU/L	57.00 ± 48.47	26.40 ± 11.80	< 0.001
ALT, IU/L	91.27 ± 97.67	16.28 ± 9.78	< 0.001
Total bilirubin, mg/dL	0.73 ± 0.56	0.57 ± 0.20	0.070
ALP, IU/L	251.48 ± 96.90	201.74 ± 71.51	0.003
GGT, IU/L	71.27 ± 65.50	53.00 ± 48.08	0.693
Proteins, g/dL	7.40 ± 0.46	7.05 ± 1.10	0.049
Albumin, g/dL	4.46 ± 0.28	4.32 ± 0.42	0.034

Values are presented as mean ± standard deviation or frequency or number (%).
BMI = body mass index, BP = blood pressure, WBC = white blood cell, CRP = C-reactive protein, AST = aspartate aminotransferase, ALT = alanine aminotransferase, ALP = alkaline phosphatase, GGT = gamma-glutamyl transferase.
[a]Continuous variables were analyzed using the *t*-test, while categorical data were analyzed using the χ^2 test or Fisher's exact test; [b]Hypertension was defined as systolic BP ≥ 95th percentile or diastolic BP ≥ 95th percentile across children of the same sex and age.

Table 2. FibroScan® results

Variables	Obese (n = 59)	Non-obese (n = 47)	P value[a]
LSM, kPa	5.81 ± 1.96	4.47 ± 0.95	< 0.001
CAP, dB/m	292.69 ± 48.29	204.15 ± 44.25	< 0.001

Values are presented as mean ± standard deviation.
LSM = liver stiffness measure, CAP = controlled attenuation parameter.
[a]Continuous variables were analyzed using the *t*-test.

LSM distribution of non-obese group

FibroScan® was used successfully in all 106 subjects (aged 5–15 years). In the non-obese control group, logarithmically converted LSM values had a relatively normal distribution, with a value of 5.5 kPa corresponding to the 95th percentile threshold. These results held true regardless of age (**Fig. 4**).

Clinical characteristics of obese children

Liver fibrosis was observed in 30 of 59 obese participants. Compared to the non-fibrosis subgroup (29 participants), the fibrosis subgroup was characterized by significantly higher age, height, weight, BMI, waist circumference, BP, and levels of AST (73.57 ± 56.00 vs. 39.86 ± 31.93 IU/L, *P* = 0.009), ALT (132.47 ± 113.88 vs. 48.66 ± 51.29 IU/L; *P* = 0.001), and γ-glutamyl transferase (106.67 ± 69.31 vs. 28.80 ± 24.26 IU/L; *P* = 0.042) (**Table 3**).

Among the NAFLD predictive indices examined in this study, the NAFLD fibrosis score showed no significant difference between the fibrosis and non-fibrosis subgroups, while
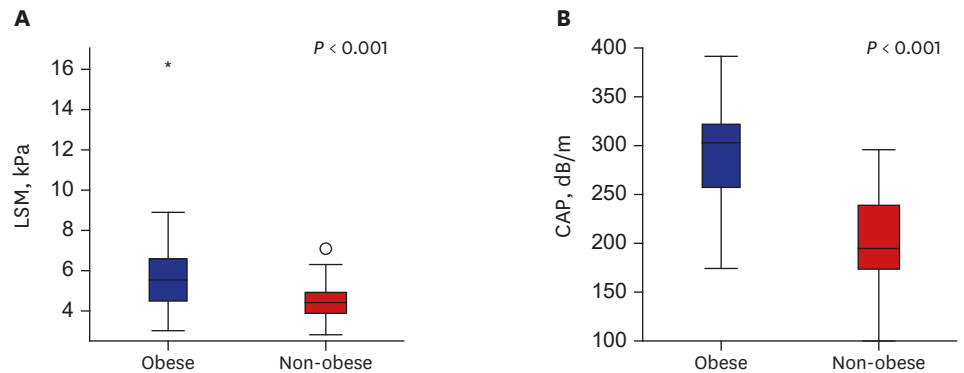
Fig. 2. Box-whisker plots of liver fibrosis and steatosis according to obesity status. (**A**) LSM values, which reflect fibrosis, and (**B**) CAP values, which reflect fat accumulation, were significantly higher in the obese group than in the control group.
Boxes denoted the interquartile range with the upper and lower horizontal edges representing the 75th and 25th percentiles, respectively. The central horizontal lines represented the medians. The vertical whisker above and below the boxes represented the range of outlying data points up to 1.5 times the interquartile range, and the star and circle represented the extreme and outlier, respectively.
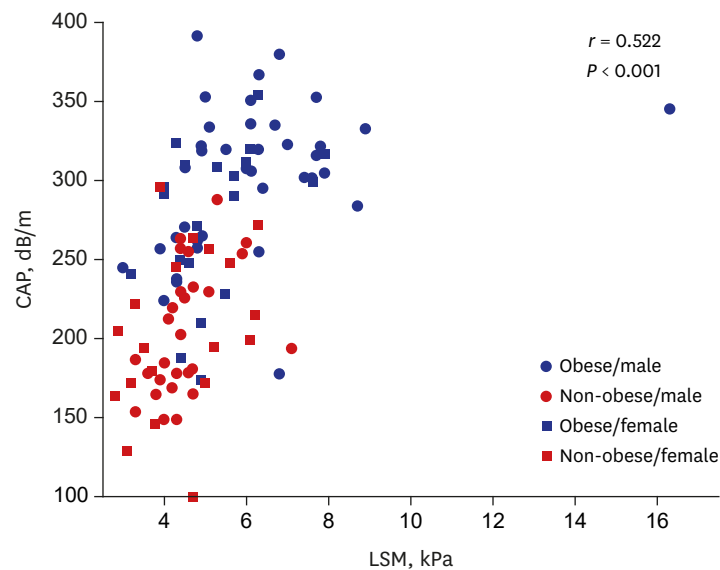LSM = liver stiffness measure, CAP = controlled attenuation parameter.

Fig. 3. Correlation of liver fibrosis with steatosis. CAP values, which reflect fat accumulation, and LSM values, which reflect fibrosis, were significantly and positively correlated across the study population regardless of obesity status or sex.
CAP = controlled attenuation parameter, LSM = liver stiffness measure.

APRI was significantly higher in the fibrosis subgroup, consistent with results previously reported in adults. The BARD score also differed significantly between the subgroups of obese children (**Table 4**).

Correlations of LSM with AST/ALT, HOMA-IR, and APRI

Among the 59 patients in the obese group, LSM showed a significant and positive correlation with not only AST levels ($r = 0.525$, $P < 0.001$), ALT levels ($r = 0.594$, $P < 0.001$), and APRI ($r = 0.480$, $P = 0.001$), which are conventional predictive indices for hepatic steatosis and fibrosis, but also with the insulin resistance index homeostasis model assessment for insulin resistance ([HOMA-IR], $r = 0.400$, $P < 0.047$) (**Fig. 5**).

Table 3. Characteristics of the obese group

Characteristics	Fibrosis (n = 30)	Non-fibrosis (n = 29)	P value[a]
Age, mon	139.43 ± 27.35	123.00 ± 29.07	0.029
Height, cm	155.83 ± 14.74	144.53 ± 15.10	0.005
Weight, kg	69.78 ± 18.81	54.07 ± 14.68	0.001
BMI, kg/m²	28.21 ± 4.05	25.31 ± 3.09	0.003
Waist circumference, cm	90.21 ± 13.19	78.40 ± 10.50	< 0.001
Systolic BP, mmHg	119.43 ± 17.89	109.45 ± 13.77	0.019
Diastolic BP, mmHg	76.00 ± 13.66	67.48 ± 9.14	0.007
Hypertension	14 (46.7)	7 (24.1)	0.071
WBC count, cells/μL	9,227.78 ± 2,462.86	8,253.85 ± 2,730.31	0.225
Hemoglobin, g/dL	13.98 ± 1.03	13.45 ± 0.97	0.094
Hematocrit, %	42.21 ± 2.94	39.75 ± 2.69	0.104
Platelets, ×10³ cells/μL	327.72 ± 36.72	315.68 ± 52.57	0.372
CRP, mg/dL	0.90 ± 1.82	0.60 ± 1.18	0.545
HbA1c, %	5.56 ± 0.24	5.50 ± 0.44	0.638
Glucose, mg/dL	99.04 ± 9.80	101.77 ± 14.99	0.450
Insulin, μIU/mL	20.33 ± 12.51	18.72 ± 10.64	0.734
HOMA-IR[b]	4.89 ± 3.35	4.49 ± 2.60	0.739
Triglycerides, mg/dL	154.22 ± 77.75	145.52 ± 58.99	0.668
Total cholesterol, mg/dL	178.34 ± 36.10	169.59 ± 38.45	0.375
HDL-cholesterol, mg/dL	47.16 ± 10.09	44.72 ± 6.40	0.185
LDL-cholesterol, mg/dL	109.58 ± 27.78	105.17 ± 25.23	0.590
AST, IU/L	73.57 ± 56.00	39.86 ± 31.93	0.009
ALT, IU/L	132.47 ± 113.88	48.66 ± 51.29	0.001
Total bilirubin, mg/dL	0.79 ± 0.38	0.66 ± 0.69	0.405
ALP, IU/L	266.03 ± 107.55	235.89 ± 83.15	0.236
GGT, IU/L	106.67 ± 69.31	28.80 ± 24.26	0.042
Proteins, g/dL	7.50 ± 0.43	7.28 ± 0.47	0.067
Albumin, g/dL	4.50 ± 0.28	4.43 ± 0.29	0.377

Values are presented as mean ± standard deviation or number (%).
BMI = body mass index, BP = blood pressure, WBC = white blood cell, CRP = C-reactive protein, HbA1c = glycated hemoglobin, HOMA-IR = homeostasis model assessment for insulin resistance, HDL = high-density lipoprotein, LDL = low-density lipoprotein, AST = aspartate aminotransferase, ALT = alanine aminotransferase, ALP = alkaline phosphatase, GGT = gamma-glutamyl transferase.
[a]Continuous variables were analyzed using the t-test, while categorical data were analyzed using the χ^2 test or Fisher's exact test; [b]HOMA-IR was computed as fasting insulin (uIU/mL) × fasting glucose (mg/dL) / 405.
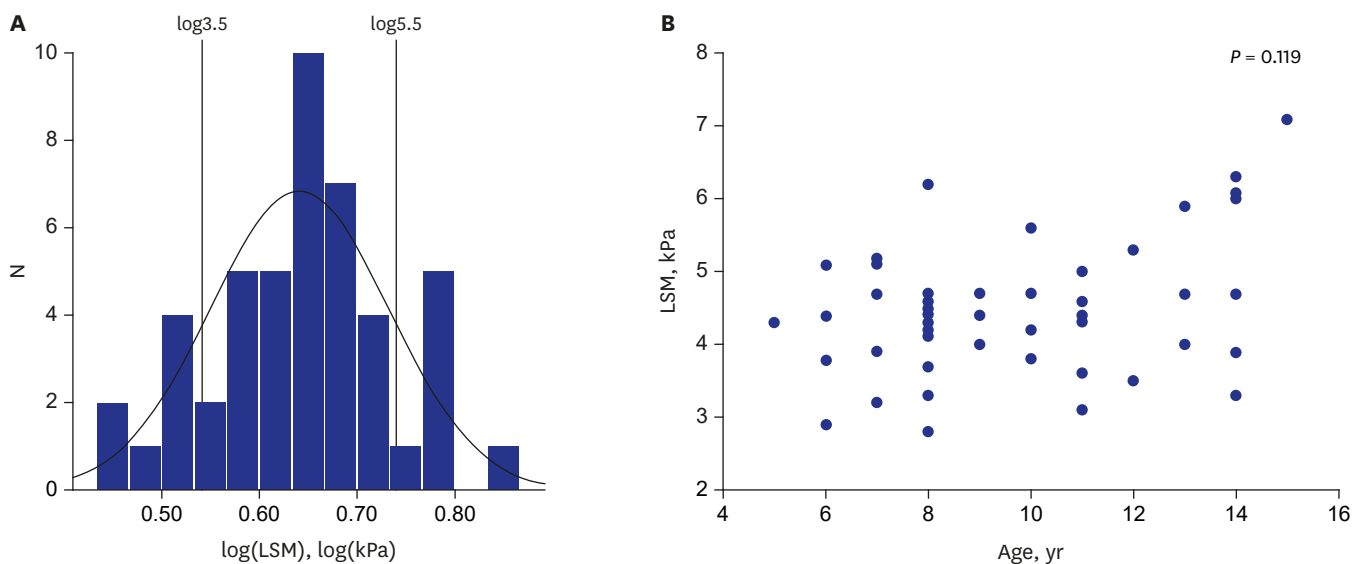

Fig. 4. Incidence of liver fibrosis in non-obese children (control group). (**A**) Logarithmically converted LSM values presented relatively normal distribution, with a value of 3.5 and 5.5 kPa corresponding to the 5th and 95th percentile thresholds, respectively. (**B**) The distribution of LSM values was independent of age (5–15 years). LSM = liver stiffness measure.
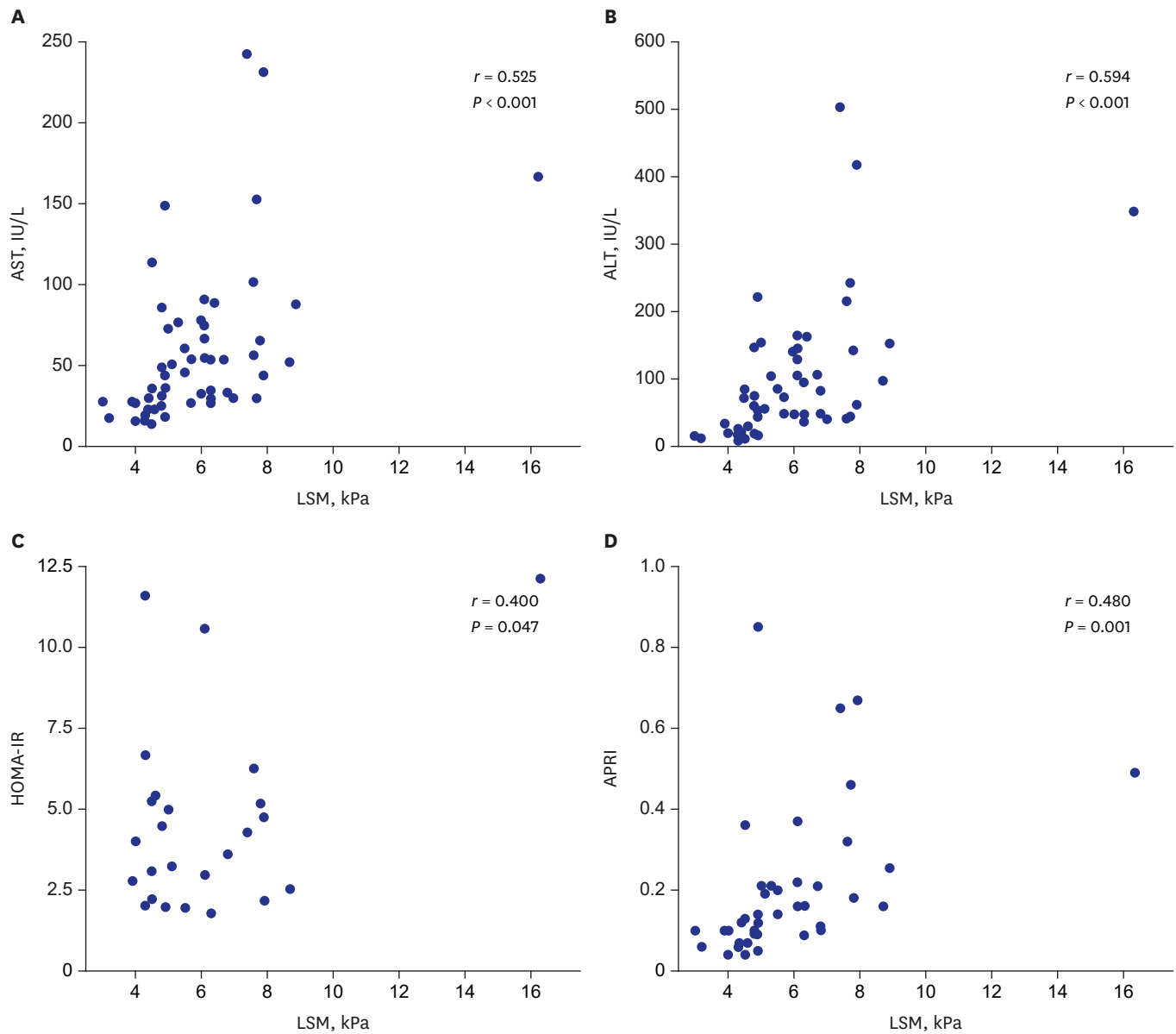
Fig. 5. Correlation of liver fibrosis with liver function and NAFLD predictive indices in obese children.
AST = aspartate aminotransferase, LSM = liver stiffness measure, ALT = alanine aminotransferase, HOMA-IR = homeostasis model assessment for insulin resistance, APRI = AST-to-platelet ratio index, NAFLD = non-alcoholic fatty liver disease.

Table 4. NAFLD predictive indices

Index	Obese (n = 59)	Fibrosis (n = 30)	Non-fibrosis (n = 29)
NAFLD fibrosis score	−3.91 ± 2.07	−3.45 ± 2.51	−4.39 ± 1.38
APRI	0.19 ± 0.18	0.27 ± 0.18[a]	0.14 ± 0.16[a]
BARD score	1.08 ± 1.06	0.67 ± 0.80[b]	1.52 ± 1.12[b]

Values are presented as mean ± standard deviation. Continuous variables were analyzed using the t-test.
NAFLD = non-alcoholic fatty liver disease, APRI = AST-to-platelet ratio index, BARD = BMI + AST/ALT ratio + diabetes mellitus, BMI = body mass index, AST = aspartate aminotransferase, ALT = alanine aminotransferase.
[a]$P = 0.032$; [b]$P = 0.005$.

DISCUSSION

FibroScan® is a non-invasive, fast, reproducible, and cost-effective test. Published studies strongly suggest that FibroScan® represents a valid test that is clinically applicable for simultaneous evaluation of hepatic steatosis (quantified as CAP) and liver fibrosis (quantified as LSM). However, such studies were mostly conducted in adults, and there is a paucity of data on the clinical suitability and reliability of FibroScan® in children. In the present study, which focused on Korean children aged 5–15 years, we clearly demonstrated the clinical applicability of FibroScan® for this population. In particular, we found a strong correlation of the FibroScan® results with blood test results such as AST and ALT, as well as with NAFLD predictive indices, indicating that FibroScan® may serve as a useful screening tool for NAFLD in obese Korean children.

In clinical practice, ALT continues to be considered the primary indicator of liver function and thus is used to screen for liver disease and evaluate disease severity.[16,17] In a recent study focused on children, Schwimmer et al.[18] reported that ALT levels were significantly correlated with the severity of liver fibrosis, but the correlation was weak. It is important not to overlook the limitations of ALT as a biomarker for liver disease. In particular, the specificity of ALT in NAFLD diagnosis is low, and this is the case even in overweight and obese children. Moreover, while NASH is more common in individuals with serum ALT levels > 80 IU/L, NASH is also observed in a considerable number of individuals with low ALT levels.[19,20] In fact, the diagnostic sensitivity of ALT for NASH was reported at only around 40%.[21] Therefore, FibroScan® examination may serve as a suitable tool for the diagnosis and grading of liver fibrosis not only in obese children with high ALT levels, but also in those with normal ALT levels.[15]

Another interesting result of our study came from the subgroup analysis (fibrosis vs. non-fibrosis) of NAFLD predictive indices among obese children. First, APRI was significantly higher in the fibrosis subgroup, mirroring patterns previously observed in normal adults, which indicates that APRI can at least partially serve as a predictive index of NAFLD in children.[22] However, because the number of subjects in our study was too low to provide adequate statistical power for the sub-group analysis, we were not able to generate an equation and provide a reference range for APRI in Korean children. Meanwhile, the BARD score also differed significantly between the subgroups, but the pattern was opposite to that previously reported in adults (i.e., a lower BARD score would indicate lower risk of fibrosis); specifically, we found a lower BARD score in obese children with fibrosis than in those without fibrosis.[23] Notably, the BARD score was designed with the expectation that the AST/ALT ratio would be at least 0.8 in individuals with liver fibrosis. However, in our study, the mean AST/ALT ratio was significantly lower than that in obese children with fibrosis, at only 0.61 (compared to 1.09 among obese children without fibrosis). This suggests that while AST/ALT ratio is a meaningful predictive index of liver fibrosis in adults, this is not true for children.

Our study demonstrated that FibroScan® can be successfully performed in children from a relatively broad age range (5–15 years). Several factors affecting the failure rate and reliability of FibroScan® measurements have been described in adults. The reported failure rate in adults was 5%–11%, and patient-related factors independently associated with FibroScan® failure in adults include female sex, high BMI, and metabolic syndrome.[24,25] Pediatric studies reported a failure rate of approximately 15%, with the highest failure rate observed in children aged under 6 years.[26] One Japanese study highlighted two important reasons for FibroScan® failure, including excessive thickness of the subcutaneous fat in obese children and the lack of

cooperation when conducting measurements in young children.[15] Thick subcutaneous fat is an important risk factor for measurement failure because the M probe signal only penetrates to a depth of 2.5 cm from the skin surface; thus, in such cases, the clinician must decide whether to reassess the patient using the XL probe, even at the cost of reduced accuracy. Moreover, because the participant has to remain still during FibroScan® imaging, young children (≤ 6 years old) may require sedation, which is a difficult decision for clinicians.

Large-scale studies have been conducted to clarify the reference values for LSM and CAP to facilitate the assessment of fibrosis and hepatic steatosis in adults with NAFLD, chronic hepatitis B, or hepatitis C, and the reported values are mostly consistent.[27,28] However, there is no consensus about reference ranges in children, especially regarding the range of LSM values and thresholds for diagnosing fibrosis in NAFLD.[26] In our study, despite the small sample size, the control group showed a relatively normal distribution for the logarithmically converted LSM, with the value of 5.5 kPa corresponding to the 95th percentile threshold, regardless of age. Therefore, we used 5.5 kPa as the reference threshold for diagnosing liver fibrosis in the participants. This is comparable to the LSM 95th percentile thresholds previously reported in healthy adults and Japanese children (5.9 kPa and 6.1 kPa, respectively).[29,30] However, given that LSM is affected by multiple factors, care is required when interpreting LSM values in obese children. Studies in adults have demonstrated that subcutaneous fat thickness, obesity, metabolic syndrome, sex, and aging affect LSM.[31,32] In particular, one study found that LSM value was overestimated to 11 kPa in healthy phantoms with overlaying fat layers of thicknesses exceeding 45 mm.[33] However, we failed to measure fat layers of the study subject, so we tried to reanalyze between groups using the propensity score matching to correct height and weight, but it was not statistically meaningful because of the low number of people studied. Another study revealed that liver fibrosis increased with central venous pressure in patients with congestive heart failure and that fibrosis increased as a secondary inflammatory response in steatohepatitis patients.[34] In the obese group, we observed significant and positive correlations of LSM with conventional predictive indices for hepatic steatosis and liver fibrosis (with AST: $r = 0.525$, $P < 0.001$; with ALT: $r = 0.594$, $P < 0.001$; with APRI: $r = 0.480$, $P = 0.001$), as well as with the insulin resistance index HOMA-IR ($r = 0.400$, $P < 0.047$), demonstrating that FibroScan®-based measurements have high reliability. Nevertheless, further study is warranted to clarify the reference ranges for LSM and CAP in children.

Despite its multiple limitations,[16] liver biopsy remains the gold standard for assessing liver inflammation and fibrosis in NAFLD, chronic hepatitis B, and hepatitis C.[20] However, non-invasive imaging and biochemical alternatives are highly desirable especially in children, who have higher risk of complications and more pronounced discomfort. Imaging approaches for evaluating steatosis and liver fibrosis in childhood NAFLD include abdominal ultrasonography, abdominal computed tomography, abdominal magnetic resonance (MR) imaging, FibroScan® (hepatic elastography), MR elastography, and MR spectroscopy.[3,34] Of these, abdominal ultrasonography is the most widely used, despite its limited capacity to quantify steatosis and fibrosis, as well as its high dependence on the examiner's skill.[4,20] Some studies reported the use of abdominal MR imaging for diagnosis and follow-up of NAFLD,[3,35] but this approach is very resource-demanding and thus cannot be applied for routine diagnosis and follow-up of pediatric steatohepatitis in the broad clinical practice. In our study, to compare with the diagnostic methods established to date, 22 of the 59 obese groups in this study were examined for abdominal ultrasonography or abdominal computed tomography, 20 and 2, respectively. Among them, 8 children were diagnosed with normal or mild fatty liver, 8 children with moderate fatty liver, and 6 children with severe fatty liver.

There was a tendency for CAP values to be higher in children with moderate or severe fatty liver than in children with normal or mild fatty liver, but there was no statistically significant difference. Interestingly, 2 of 4 children identified as normal in abdominal ultrasonography or abdominal computed tomography were found to have a CAP value greater than 272 dB/m corresponding to the 95th percentile threshold in the control group. Therefore, FibroScan® may be a more sensitive tool to screen for hepatic steatosis. FibroScan® has unique advantages in this regard, providing a non-invasive, rapid, reproducible, cost-effective, and radiation-free solution that can be easily implemented for routine diagnosis and follow-up of NAFLD in obese children.[15] Based on our clinical experience and on available literature evidence, we support the wide adoption of FibroScan® in the clinical practice of pediatricians.

This study had several limitations. First, because we were unable to perform the gold standard test of liver biopsy to evaluate liver inflammation and fibrosis among the participants, we could not define LSM and CAP thresholds corresponding to certain severity levels of fibrosis and steatosis, respectively. Although children had liver diseases such as biliary atresia, autoimmune hepatitis, and Wilson disease, not NAFLD, one study of 33 children who conducted both liver biopsy and FibroScan® suggested an LSM cut-off value of 5.4 kPa to diagnose liver fibrosis.[36] Additionally, although adults were the target in several studies, LSM cut-off values in the diagnosis of liver fibrosis through liver biopsy in NAFLD patients were 5.5 kPa, 5.9 kPa, and 5.35 kPa, respectively.[37-39] This results were similar to LSM cut-off value of 5.5 kPa for classifying fibrosis in our study. Despite the limitation of failing to perform liver biopsy, we identified strong correlations between LSM and relevant biochemical markers, which, in the future, may help develop adequate cut-off values for diagnosing and monitoring NAFLD in children. Second, some of the non-obese children were non-fasting at the time of sampling, so a few biochemical variables suggested a limitation in comparing obese with non-obese groups because there were results that required fasting, such as glucose measurements. Third, the study sample was not representative of the general population and was restricted to children who visited our hospital as inpatients or outpatients. Therefore, we should be careful not to overlook the possibility that FibroScan® results and conclusions drawn for the non-obese control group may not immediately apply to healthy children. Nevertheless, the focus of our study was to investigate differences between obese children and non-obese children in terms of FibroScan® results and liver-related biochemical marker, with a particular focus on determining whether FibroScan® could serve as a valid test for assessing liver fibrosis and steatosis in obese children. In this context, our results strongly support the usefulness of FibroScan® in pediatric patients.

In conclusion, FibroScan® has unique advantages including non-invasiveness, speed, and reproducibility, which confirms the feasibility and validity of implementing FibroScan®-based clinical screening for hepatic steatosis and fibrosis in Korean children. Importantly, FibroScan® screening allows the examiner to simultaneously quantify the extent of steatosis and the extent of fibrosis. Moreover, the FibroScan® results show strong correlation with AST, ALT, and APRI, which are used as predictive indices of NAFLD, suggesting that FibroScan® has high reliability.

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