The Results of ARFI (Acoustic Radiation Force Impulse) Elastographic Assessment of Liver Aspect and NAFLD (Non-alcoholic Fatty Liver Disease) in Pediatric Obese Patients Compared to Normal-weight Children

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Background: The incidence of obesity has dramatically increased in the last few years, and associated disorders such as non-alcoholic fatty liver disease (NAFLD) constitute a serious threat. The objective of our study was to assess the liver aspect of obese children and adolescents by real-time elastography, ARFI-technique, compared to the liver aspect of normal-weight children.

Methods: Eighty-six children, aged 3–18 years, admitted to the County Emergency Clinical Hospital of Tîrgu Mureş between 15 September 2010 and 15 April 2012, were recruited for the study. They were included in two groups: 39 overweight/obese children and 47 normal-weight healthy controls. We evaluated the liver-tissue elasticity by measuring the Shear Wave Velocity (SWV), globally and separately for segments 1 and 8 in order to detect possible differences beetween them, knowing that the caudate-lobe has it's own vasculature; we also evaluated biochemical parameters (transaminases, etc). Correlations between SWV and laboratory tests were established using non-parametric Spearman correlation test.

Results: In healty children in the 1st segment SWV was 1.012±0.31 m/s, smaller than in the 8th segment, 1.342±0.32 m/s ($p = 0.0316$). For obese children, SWV was higher in the 8th segment 1.982±0.85 m/s compared to the 1st segment 1.325±0.27 m/s ($p < 0.0001$). Globally in obese children, the SWV was 1.746±0.49 m/s, significantly higher than in healthy children, 1.080±0.27 m/s ($p = 0.0023$). Positive statistical correlations have been established between SWV and aspartate-aminotransferase in obese ($r = 0.61$, Pearson correlation $p = 0.025$), with no statistically significant differences for other laboratory findings.

Conclusion: Elastographic evaluation of liver alterations in obesity by ARFI-method shows higher SWV, which could be translated in fibrosis and necroinflammatory activity. According to our study, these alterations in liver tissue affect mainly the right lobe of the liver.

Keywords: ARFI, elastography, NAFLD, obesity, child

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Introduction

Obesity and visceral adipose tissue affect the liver and hepatocytes. Chronic lipid supply exceeding the metabolic ability of the liver may induce hepato-cellular injury. Non-alcoholic fatty liver disease (NAFLD) is a disease mostly influenced by obesity [1].

The prevalence of NAFLD is increasing in the last few years, probably because the incidence of overweight and obese patients has also increased dramatically, in the pediatric population as well [2]. The visceral adipose tissue secretes free fatty acids (FFAs) and hormones (adipokines), and thus seems to play a major role in the development of NAFLD [1].

NAFLD represents a broad spectrum of conditions ranging from fatty liver, which in general follows a benign nonprogressive clinical course, to steatohepatitis or NASH (non-alcoholic steatohepatitis), a more serious form of NAFLD [3].

NAFLD is the most common cause of elevated liver enzymes; underlying pathophysiological mechanisms associated with NASH-progression are scarcely understood [4]. Regardless of its pathway, the final result of injury in chronic liver disease is fibrosis, which may progress to cirrhosis and end-stage liver disease (liver failure) [3].

Morbid conditions associated to NAFLD are diabetes (up to 30%), hipertryglicerides (almost 50%) and obesity (65%) [5].

The natural evolution of NAFLD in adults presents a slow progression to cirrhosis, while in children there are no specific data. A study performed in the USA on 742 children revealed in 13% of cases steatosis, in 23% steatohepatitis, in 9% severe fibrosis and cirrhosis. In 1984 the first case of cirrhosis due to non-alcoholic steatohepatitis was reported (a diabetic 15 year-old girl, with obesity since the age of four).

The diagnosis of NAFLD in children follows a usual pattern: 11–13 year-old overweight or obese boys, although almost 10% of the cases do not respect this pattern [7].

Clinically there are no symptoms or abdominal pain, obesity is associated with achantosis nigricans, with or without hepatomegaly.

In 2003, Sanyal et al. reported upper digestive haemorrhage, ascites, oedema, pruritus in less than 5% of children with NAFLD, abdominal pain in 35%, while more than 60% of pediatric patients complained of fatigue, and

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25–30% were asymptomatic.

Laboratory tests show a moderate elevation of transaminases (ALT more than AST); slight increase of γGT, alcaline phosphatase (AF); normal levels of bilirubine, albumine and normal Quick time; hyperlipemia with hyper-trygliceridemia; low titre of non-specific auto-antibodies (SMA); high level of insulin and insulin resistance; normal blood sugar; low plasma adiponectin; no other liver diseases with steatosis (Wilson disease, HCV hepatitis, autoimmune hepatitis) [6].

Imagistic aspects: current non-invasive imaging methods cannot characterise properly non-alcoholic steatohepatitis with/without fibrosis, although abdominal ultrasound, computed tomography and magnetic resonance imaging bring useful information. Histological examination remains the gold standard for diagnosis, but liver biopsy requires specific criteria (low age, <10 years, hepatosplenomegaly, very high levels of transaminases, severe insulin-resistance, non-specific autoantibody, inconclusive biochemical tests for Wilson's disease, associated liver diseases – viral hepatitis, α1 antitrypsin-defficiency, hypothalamic disorders, severe family NAFLD history, planned drug-therapy [6].

Fatty infiltration of the liver may be diffuse or local. Diffuse steatosis is characterized by increased tissue-echogenicity, with attenuation of ultrasound transmission in depth, while the adjacent renal cortex may appear hypoechogenic, falsely transsonic compared to liver [8,9,10].

In parceling steatosis, fat deposits appear only in certain areas of the liver parenchyma, there are hyperechogenic areas forming large or small beaches, alternating with areas of healthy parenchyma. Fat-loaded areas are hyperechogenic, as normal liver tissue appears falsely hypoechogenic [10,11].

In a state of an increased interest in finding a non-invasive alternative method to hepatic biopsy to evaluate the degree of hepatic fibrosis, ARFI technology is emerging as a noninvasive imaging technique, allowing a quantitative assessment of liver stiffness, by using acoustic wave impulses. Only preliminary studies have been published so far on ARFI technology, reporting data on adult population [12,13]. These methods have not been evaluated in pediatric patients. Some studies are under development, but data are either insufficient or refer specifically to transient elastography [14].

Our study aimed to assess the liver aspect of obese children and adolescents by real-time elastography – ARFI technique, compared to the liver aspect of normal-weight children and also the presence of signs and measurements suggestive for NAFLD and/or steatohepatitis.

Material and methods

Consecutive children, aged between 3–18 years, hospitalized in the 1st Pediatric Clinic of Tîrgu Mureș between 15 September 2010 and 15 April 2012 were considered eligible for the study. The following groups were recruited: normal-weight healthy controls (without any changes of liver echostructure on conventional 2B mode ultrasonography, normal transaminases, normal AF) and children with overweight or obesity, as follows: patients with BMI-for-age between the 85th and 95th percentiles were considered overweight, while those above the 95th percentile were considered obese (according to World Health Organization standards) [15].

Children with chronic viral hepatitis, autoimmune hepatitis, α1 antitrypsin deficiency, Wilson's disease, hepatomegaly of other etiology, were excluded from the study.

The following laboratory tests were performed: alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transpeptidase (GGT), alkaline fosfatase (AF), total cholesterol, triglycerides, total bilirubine. Height (kg) and weight (cm) were used to calculate the Body Mass Index (kg/m^2) (BMI). Medium Upper Arm Circumference (MUAC) was measured at the mid-point between the tips of the shoulder and elbow, using a tape measure calibrated in centimeters and Tricipital Skin Fold (TSF) was measured in the posterior upper arm, using a thickness caliper, as additional surrogate measures for nutritional status.

A single examiner performed US elastography with an ultrasound machine Siemens S 2000, equipped with a transducer of 4.1 MHz. Real-time Acoustic Radiation Force Imaging (ARFI) – Virtual Touch (VT) tissue quantification technology (Siemens) was used for fibrosis quantification. Global shear wave velocity SWV (m/s) was measured.

Informed consent was obtained from parents or legal tutors in compliance with the principles of the Helsinki Declaration, at the moment of admission to the hospital. The study was approved by the local Committee of Ethics of the University of Medicine and Pharmacy of Tîrgu Mureș.

Statistical analysis: data were analyzed with Microsoft Office Excel 2010, and the statistical analysis was done with Graph Pad Prism 5 and Graph Pad InStat 3.06. Continuous variables were expressed as median ± standard error of mean (SEM) or standard deviation (SD). Comparisons between the groups were assessed using parametrical or non-parametrical tests, depending of the distribution of the variables (Student t test and ANOVA); p values of < 0.05 were considered statistically significant. Correlation between median SVW of different groups and the other study variables (transaminase and parameters of nutritional status) was assessed with Pearson's correlation coefficient (r).

Results

Eighty-six of 91 consecutive children, who were screened for the study, were enrolled, while 5 met at least one exclusion criteria.

Forty-seven of the 86 children were healthy controls, with normal weight and BMI-for-age, and 39 were overweight or obese.

Table I. Characteristics of the study groups – demographic and anthropometric aspects

	Healthy controls n = 47	Overwight/obese n = 39
Age (years) mean ± SD (range) median	 7.57 ± 4.26 (3–17) 6.08 years	 10.16 ± 4.66 (3–18) 10 years
Males (%)	26 (55%)	25 (64%)
BMI-for-age z-score, mean ± SD (range)	2.92 ± 0.70 (1.54–4.55)	0.31 ± 0.76 (-1.27–1.40)
MUAC z-score mean ± SD (range)	4.48 ± 1.63 (2.22–8.05)	-0.43 ± 0.82 (-1.5–1.46)
TSF z-score mean ± SD (range)	4.04 ± 1.35 (2.57–7.58)	-0.06 ± 0.94 (-1.5–1.39)

Demographic and anthropometric characteristics of the groups are presented in Table I.

In the overweight or obesity group males were predominant and children were older, consecutively, as expected, BMI-for-age, MUAC and TSF z-scores were higher.

A descriptive statistical analysis regarding the biochemical test results is presented in Table II. ALT and AST values were lower in the control group, compared with the overweight or obesity group. Data of elastographic measurements (SWV) are also presented in Table II.

In healthy children in the 1st segment Shear Wave Velocity was 1.012±0.31 m/s, smaller than in the 8th segment, 1.342±0.32 m/s (p = 0.0316), as presented in Figure 1.

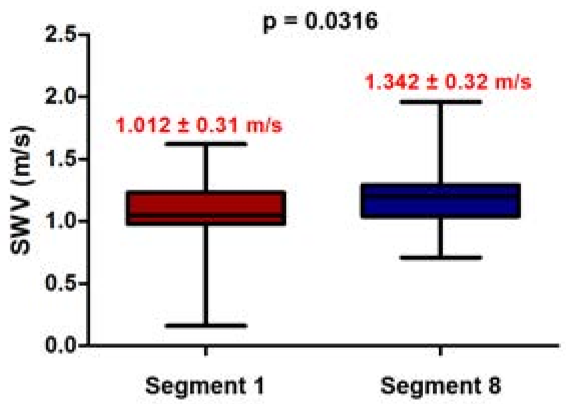

Fig. 1. Smaller SWV in the 1st than in the 8th liver segment for healthy children in normal conditions, caudate lobe is "softer"; p = 0.0316

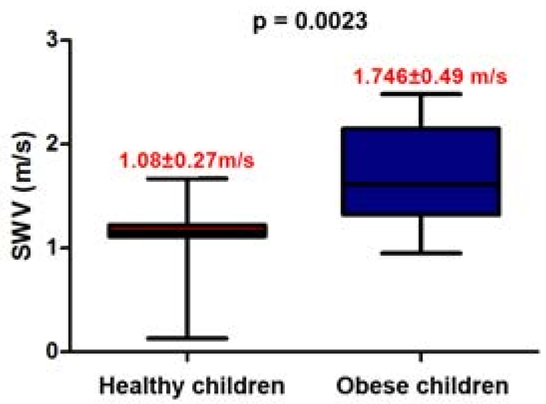

Fig. 3. Global SWV in obese – higher than in healthy children, p = 0.0023

Table II. Biochemical characteristics and elastographic measurement values of the studied groups

Variable mean ± SD	Healthy controls n = 47	Overwight/obese n = 39
ALT (UI/l)	18.2 ± 8.5	31.9 ± 15
AST (UI/l)	23.1 ± 12.7	34.8 ± 15.6
GGT (unit)	35.4 ± 11	39.80 ± 7.79
Total cholesterol (mg/dL)	131.6 ± 22.9	167.6 ± 46.3
Triglycerides (mg/dL)	46.5 ± 22.1	110.6 ± 22.4
Total Bilirubin (mg/dL)	0.5 ± 0.1	0.99 ± 0.36
Direct Bilirubin (mg/dL)	0.20 ± 0.11	0.32 ± 0.2
SWV (m/s)		
Global	1.08 ± 0.27	1.746 ± 0.49
1st segment	1.012 ± 0.31	1.325 ± 0.27
8th segment	1.342 ± 0.32	1.982 ± 0.85

For obese children SWV was higher in the 8th segment 1.982±0.85 m/s compared to the 1st segment 1.325±0.27 m/s, with a statistically significant difference ($p < 0.0001$), as presented in Figure 2.

Globally in obese children SWV was 1.746±0.49 m/s, significantly higher than in healthy children 1.08±0.27m/s ($p = 0.0023$), suggesting that hepatic fatty infiltration may decrease hepatic elasticity hence the increased SWV, as seen in Figure 3.

Positive statistical correlations have been found between SWV and aspartate-aminotransferase in obese ($r = 0.61$, Pearson correlation $p = 0.025$), as seen in Figure 4.

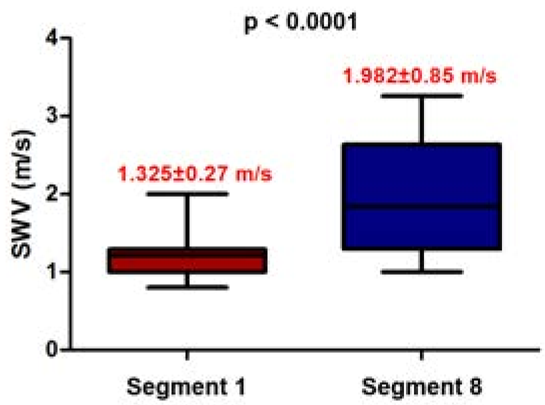

Fig. 2. Smaller SWV in the 1st than in the 8th liver segment for obese children; p = 0.0001

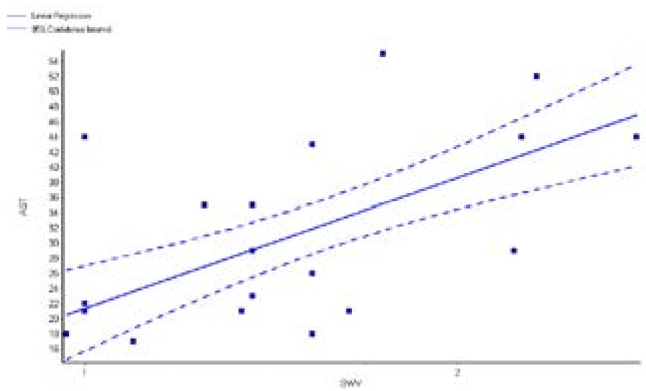

Fig. 4. Correlations between SWV and aspartate-aminotransferase in obese children

Correlations between global and segmental SWV and BMI, TSF, MUAC and ALT levels were tested for each group, but they were not statistically significant.

Discussion

Although in the literature in NAFLD a moderate increase of transaminase is reported, with ALT increasing more than AST, in this study we found a different situation; AST was more elevated than ALT, but without significant difference between them. We found a significant correlation between AST levels and SWV in children with overweight and obesity.

There are more and more clinical applications of the ARFI technology in adult studies and practice. In the literature there are only a limited number of studies about ARFI in children, which explaines the motivation of our study.

In the group of children with overweight and obesity, SWV values were significantly higher than in the healthy group, suggesting, as other authors reported, that liver steatosis results in higher values of the SWV, although there are studies which state that hepatic steatosis would not influence the degree of hepatic fibrosis [16]. Such controversial results are explained by a limited number of studies, most of them including adult patients, thus liver parenchyma changes in obese children need further research.

Other studies have also looked for correlations of SWV with fibrosis, liver steatosis and liver necro-inflammatory activity [16,17]. Although we assessed adiponectin levels and other biochemical parameters, some tests are still in course, values are not avaible yet, so the interpretation of these results is not the aim of this paper.

In a small number of cases in our cohort, we encountered erroneous recordings due to the marked attenuation of the signal in obese children and excessive movement of the tissue (e.g.: child mouvement, crying, heart beats) which required reexamination and recalibration.

Our study has several limitations: the limited number of children and especially the lack of histological liver evaluation.

Conclusions

SWV measurement by ultrasound elastography (ARFI method) in children with liver disease in the setting of overweight and obesity appears to correlate directly with the histological degree of fibrosis. ARFI represents a safe non-invasive method of evaluation of hepatic fibrosis in a pediatric population, adding useful information to the clinical and biochemical parameters.

Further studies are needed in order to establish whether US elastography may be able to replace hepatic biopsy as a non-invasive alternative investigation in the diagnosis of pediatric NAFLD in obesity.

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