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ORIGINAL ARTICLE

Enhanced liver fibrosis (ELF) test in obese children with ultrasound-proven liver steatosis

Patrycja Szybowska [1], Malgorzata Wojcik [2], Jerzy B. Starzyk [2], Krystyna Sztefko [1]

1 Department of Clinical Biochemistry, Institute of Pediatrics, Jagiellonian University Medical College, Krakow, Poland.
2 Department of Pediatric and Adolescent Endocrinology, Chair of Pediatrics, Institute of Pediatrics, Jagiellonian University Medical College, Krakow, Poland.

Correspondence to: Szybowska Patrycja, MSc.
Department of Clinical Biochemistry
Institute of Pediatrics, Jagiellonian University Medical College
ul. Wielicka 265, 30-663 Krakow, Poland.
tel/fax: +48 12 658 06 81; e-mail: patrycja.szybowska@uj.edu.pl

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Abstract

OBJECTIVE: Non-alcoholic fatty liver disease (NAFLD) in obese children is a diagnostic challenge. The enhanced liver fibrosis test (ELF) based on the combination of serum concentration of hyaluronic acid (HA), aminoterminal propeptide of type III procollagen (PIIINP), tissue inhibitor of matrix metalloproteinase type 1 (TIMP-1) was developed as a noninvasive diagnostic tool for estimation of degree of liver fibrosis. The aim of our study was to investigate the performance of ELF test in obese children with ultrasound-proven steatosis in order assess the possibility of early detection of fibrotic changes in liver structure.

MATERIAL AND METHODS: 58 obese (BMI >95th percentile) children, 27 male (mean age 13.9±2.65 years) and 31 female (mean age 13.82±2.64 years). Based on the liver ultrasound (US) examination results two groups of obese children were studied: group with steatosis (N=20, 8/12 M/F, mean age 14.2±1.90 years, BMI 32.9±5.60 kg/m^2) and group with normal liver US (n=38, 19/19 M/F, mean age 13.7±2.94 years, BMI 30.4±4.67 kg/m^2). Serum activity of aminotransferases (AST, ALT) and lactate dehydrogenase (LDH), and γ-glutamyl transpeptidase (GGT), and ELF test (HA, PIIINP, and TIMP-1 were analyzed.

RESULTS: Children with liver steatosis presented with significantly higher AST (34.1 vs. 25.6 U/L), ALT (43.4 vs. 32 U/L), LDH (427.5 vs. 361.3 U/L), GGT (30.7 vs. 18.9 U/L). The ELF test value was also significantly higher in that group (8.98 vs. 8.49). Nevertheless no combination of measured parameters with ELF test value show better diagnostic value for differentiation between children with and without steaotosis.

CONCLUSION: ELF test cannot be used for assessment of steatosis in obese children.

INTRODUCTION

Obesity is a growing worldwide problem in general population so prevention from serious consequences at early stages of this condition become more and more important (Moya 2008; Ogden *et al.* 2014). One of its consequences is the increasing incidence of nonalcoholic fatty liver disease (NAFLD) (Alisi *et al.* 2009; Mencin & Lavine 2011), which affects 2.6–9.8% of children and ado-

lescents in general population (Tominaga *et al.* 1995; Franzese *et al.* 1997; Schwimmer *et al.* 2006), and up to 74% obese individuals (Patton *et al.* 2006; Papandreou *et al.* 2007; Dunn & Schwimmer 2008; Manco *et al.* 2008). NAFLD is wide spectrum of liver pathology ranging from relatively benign hepatocellular steatosis to irreversible liver cirrhosis, caused by an accumulation of triglyceride (TG) in liver cells. It is defined by hepatic fat infiltration of more than 5% hepatocytes in the absence of excessive alcohol intake, viral, autoimmune and drug-induced liver disease (Clark *et al.* 2002). Recently, NAFLD became the main cause of chronic liver disease in children (Day 2011; Matthiessen *et al.* 2008; Ji 2008). There is no clear clinical features that could indicate NAFLD definitely. Moreover, distinguishing simple steatosis from more advances forms such as nonalcoholic steatohepatitis (NASH), nonalcoholic hepatic fibrosis and nonalcoholic cirrhosis in affected patient is frequently needed. NASH may progress towards cirrhosis also in pediatric population thus it is necessary to assess the stage of liver fibrosis (Powell *et al.* 1990; Molleston *et al.* 2002; Feldstein *et al.* 2009; Roberts 2007; Jankowska *et al.* 2007; Jonas *et al.* 2005). Nowadays percutaneous liver biopsy as the gold standard is commonly used for NAFLD diagnosis (Takahashi & Fukusato 2010; Neuschwander-Tetri *et al.* 2010; Straub & Schirmacher 2010). However, this reference method has limitations resulted from biopsy sampling variability and invasive nature of the procedure (Bedossa *et al.* 2003; Maharaj *et al.* 1986; Cadranel *et al.* 2000; Ratziu *et al.* 2005; Rockey *et al.* 2009). Due to these shortcomings the development of noninvasive diagnostic methods for staging the degree of fibrosis should be a priority, especially in pediatric population. It is even more important, as NALFD in pediatric patients differs histologically from one observed in adults (Schwimmer *et al.* 2005; Ko *et al.* 2009).

The enhanced liver fibrosis (ELF) test, based on the combination of three direct serum markers concentration measurement: hyaluronic acid (HA), amino-terminal propeptide of type III procollagen (PIIINP), and tissue inhibitor of matrix metalloproteinase type 1 (TIMP-1), is a noninvasive method designed for detection of liver fibrosis in subjects with chronic liver disease caused by different factors. However, evaluation of this test in children was performed only in a limited number of studies (Nobili *et al.* 2009 "a"; Nobili *et al.* 2010; Iacobellis *et al.* 2006). The aim of our study was to investigate the performance of ELF test in obese children with ultrasound-proven steatosis in order to check the usefulness of this test for early detection of fibrotic changes in liver structure.

MATERIALS AND METHODS

A total of 58 obese children, 27 male (mean age 13.9±2.65 years, BMI 31.48±4.25 kg/m^2) and 31 female (mean age 13.82±2.64 years, BMI 31.05±5.81 kg/m^2), treated in the University Children's Hospital in Cracow, Poland, were included to the study. Obesity was defined taking into account body mass index (BMI) value higher than the 95th percentile adjusted for age and sex of the child. Out of 58 patients 34% of children had steatosis proven in ultrasound examination (US). Based on the abdominal US results two groups of obese children were studied: group I (N=20, 8/12 M/F, mean age 14.2±1.90 years, BMI 32.9±5.60 kg/m^2) with US proven steatosis and group II (n=38, 19/19 M/F, mean age 13.7±2.94 years, BMI 30.4±4.67 kg/m^2) with normal liver US. In all patients fasting blood samples were taken during a planned visit in hospital. Based on medical history alcohol consumption, the presence of autoimmunologic, drug-induced or virus-induced liver diseases has been excluded.

Serum activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), and γ-glutamyl transpeptidase (GGT) were analyzed in fresh serum samples by dry chemistry methods (VITROS® 5.1 FS, Ortho Clinical Diagnostics). For component of ELF test determination serum samples eres stored at –20 °C until assayed. HA, PIIINP, and TIMP-1 concentrations were measured separately on ADVIA Centaur XP (Siemens Healthcare Diagnostic Inc. Tarrytown, New York USA) based on immunochemiluminescence method. The concentration of HA, PIIINP and TIMP-1 were used for calculation of ELF test value using following formula: ELF=2.278+0.851 ln (HA)+0.751 ln (PIIINP)+0.394 ln (TIMP-1) according to the manufacturer instruction.

Statistical analysis

The results were expressed as the mean values with the standard deviation (SD). Student's *t*-test or Mann-Whitney *U*-test were used for between groups comparison The level of statistical significance was established at $p<0.05$. The areas under the ROC curves (AUROCs) were estimated for each enzyme measured, HA, PIIINP and TIMP-1 and ELF test. The data were analyzed using Statistica data analysis software system version 10 (StatSoft, Inc. (2011), Krakow, Poland).

RESULTS

Among all children studied only in six children AST activity was above normal, in another 13 ALT activity was above reference range, while in nine children increased GGT activity was observed. Although higher mean values of AST, ALT and GGT activity were noted for boys as compared to girls, no significant differences in the mean values of all measured parameters between boys and girls were found.

In obese children with steatosis confirmed by US the mean values of all enzymes activities were significantly higher as compared to the mean values obtained in obese children without steatosis (Table 1). However, significantly higher AST and ALT activity in boys with

steatosis (p<0.002 and p<0.001, respectively) but not in girls with steatosis as compared to boys and girls without steatosis were noted. Although higher mean values of LDH activity in boys and girls with steatosis were observed as compared to boys and girls without steatosis the difference were not statistically significant. The mean values of GGT activity in boys (p<0.001) and girls (p<0.02) with steatosis were significantly higher in patients with steatosis as compared to patients without steatosis.

Among the ELF test components, the mean concentration values of HA and TIMP-1 were significantly higher in children with steatosis as compared to children without steatosis (p<0.003 and p<0.02, respectively), whereas no significant difference was noted for PIIINP level. Nevertheless, such differences between patients with and without steatosis were seen only for boys (p<0.002, both for HA and TIMP-1) but not for girls. Significantly higher mean value of ELF test was observed for children with steatosis as compared to children without steatosis (p<0.005) (Table 2). Similar data were obtained for boys but not for girls.

Cut-off values for liver enzymes, HA, PIIINP, TIMP-1 and ELF test value as well as area under ROC curves (AUC), sensitivity, specificity, positive and negative predictive values are presented in Table 3. All parameters measured, excluding LDH, showed similar specificity but low sensitivity. No combination of measured parameters with ELF test value show better diagnostic value for differentiation between children with and without steatosis.

DISCUSSION

Invasiveness of liver biopsy used as a gold standard to assess degree of liver fibrosis is the main reason for searching new, more acceptable methods and markers. In practice noninvasive evaluation, like imaging techniques and liver enzyme measurement, is performed to confirm the diagnosis of fatty liver disease.

Tab. 1. The liver enzymes activity in obese children in relation to gender and present of steatosis.

Enzyme activity (U/L)	All children		Boys		Girls	
	With steatosis	Without steatosis	With steatosis	Without steatosis	With steatosis	Without steatosis
AST	34.1±12.5	25.6±6.7	39.3±13.4	25.8±4.6	30.7±11.1	25.5±8.4
	p<0.002		p<0.002		NS	
ALT	43.4±22.6	32.0±12.1	53.5±22.8	32.0±11.1	37.5±21.2	31.9±13.2
	p<0.006		p<0.001		NS	
LDH	427.5±103.2	361.3±68.0	395.7±73.1	353.1±41.2	447.4±118.5	370.4±91.3
	p<0.04		NS		NS	
GGT	30.7±12.7	18.9±7.1	41.9±12.2	20.1±9.0	25.1±8.8	17.5±4.1
	p<0.001		p<0.001		p<0.02	

AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; GGT, γ-glutamyl transpeptidase; NS, not significant

Tab. 2. The concentration of hyaluronic acid (HA), aminoterminal propeptide of type III procollagen (PIIINP), and tissue inhibitor of matrix metalloproteinase type 1 (TIMP-1) as well as ELF test in obese children in relation to gender and present of steatosis.

ELF test	All children		Boys		Girls	
	With steatosis	Without steatosis	With steatosis	Without steatosis	With steatosis	Without steatosis
HA	18.2±8.4	12.1±6.2	20.4±10.6	12.0±6.4	16.7±6.7	12.2±6.2
	p<0.003		p<0.02		NS	
PIIINP	19.4±8.9	17.0±8.2	19.0±9.7	17.0±8.2	19.6±8.7	16.9±8.5
	NS		NS		NS	
TIMP-1	246.1±35.5	220.8±38.3	266.6±41.7	225.2±36.6	232.4±23.7	216.5±40.4
	p<0.02		p<0.02		NS	
ELF	8.98±0.61	8.49±0.57	9.07±0.79	8.51±0.44	8.94±0.48	8.48±0.69
	p<0.005		p<0.04		NS	

HA, hyaluronic acid; PIIINP, aminoterminal propeptide of type III procollagen; TIMP1, tissue inhibitor of matrix metalloproteinase type 1; NS, not significant

Tab. 3. Cut-off values, area under ROC curves, sensitivity, specificity, positive and negative predictive values for liver enzymes, hyaluronic acid (HA), aminoterminal propeptide of type III procollagen (PIIINP), tissue inhibitor of matrix metalloproteinase type 1 (TIMP-1) and ELF test.

	Cut-off	AUC	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
AST (U/L)	33.2	0.704	50.0	92.1	76.9	77.8
ALT (U/L)	55.9	0.670	35.0	94.7	77.8	73.5
LDH (U/L)	393.7	0.692	61.5	84.2	72.7	76.2
GGT (U/L)	26.9	0.804	66.7	91.3	83.3	80.8
HA (ng/ml)	19.9	0.742	40.0	92.1	72.7	74.5
PIIINP (ng/ml)	33.0	0.586	15.0	94.7	60.0	67.9
TIMP1 (ng/ml)	276.0	0.695	15.0	97.4	75.0	68.5
ELF	9.36	0.720	35.0	94.7	77.8	73.5

AUC, area under receiver operating curve; PPV, Positive Predictive Value; NPV, Negative Predictive Value

The increasing prevalence of obesity not only in adults but also in children and adolescent is the main reason for searching new markers of early detection of liver steatosis. Evaluation of hepatic steatosis in children is usually performed by USG, however interpretation of USG results is very difficult, especially in obese children (Shannon *et al.* 2011). Thus, indirect tests and/or new test for assessment of the presence of liver fibrosis are used for clinical diagnosis.

One of the predictors of NAFLD, particularly of NASH, routinely measured in obese patients is increased activity of AST and ALT (Papandreou *et al.* 2007). Both enzyme are under influence of many pre-analytical factors like non-fasting samples, physical activity, hemolysis, freeze/thaw serum cycle, etc. It is known that in large number of children with NAFLD and NASH serum AST and ALT activities can be within reference range (Shannon *et al.* 2011; Angulo 2002; Mofrad *et al.* 2003). It was shown that increased activities of serum AST and GGT (Patton *et al.* 2008) or only AST activity can be not only a significant predictors of NAFLD but also it correlate with the stage of fibrosis so that might be use to distinguish significant fibrosis from no or mild fibrosis (Carter-Kent *et al.* 2009). In the present study significantly higher AST, ALT, LDH and GGT activity in children with US proven steatosis as compared to children without steatosis has been confirmed. However, when sex was taken into consideration it appeared that significantly higher AST and ALT activity was confirmed only for boys with steatosis but not in girls. This could be in agreement with the hypothesis that estrogen can be potentially liver-protective and androgens may aggravate NASH (Lobanova *et al.* 2009; Xu *et al.* 2004). No such differences were observed for GGT, as this enzyme was significantly higher both in boys and in girls with US proven steatosis. It was found that mildly elevated GGT activity does not reflect the severity of steatosis or fibrosis in patients with NAFLD (Shannon *et al.* 2011).

The ELF test is one of noninvasive tests used for assessing liver fibrosis stage which could be performed in children. This test includes a measurement of three extracellular matrix components: HA, PIIINP and TIMP-1. In the present study, the concentration of two ELF test components, HA and TIMP1, were significantly higher in obese children with steatosis as compared to obese children without steatosis. It was shown that HA, the most investigated direct marker for liver fibrosis, is a good marker to predict liver fibrosis in children with the cut-off value for NAFLD 19.1 ng/ml and AUC 0.672 (Lebensztejn *et al.* 2011). In the present study the cut off value for discriminating obese children with steatosis and obese children without steatosis was established at 19.9 ng/ml with AUC 0.742.

According to the published studies, the ELF test can be a good predictor of hepatic fibrosis not only in adults patients but also in children: suggested by Nobili *et al.* cut off value for ELF test in children was established at the value of 10.51 (Nobili *et al.* 2009b). Our cut off value of ELF test for discriminating obese children with and without steatosis was set up at 9.32, with AUC 0.720. Similar to AST and ALT activity, also for ELF test values the difference between obese boys and obese girls has been observed. Significantly higher ELF test value was found for obese boys with steatosis as compared to obese boys without steatosis.

The combination of single tests results did not improved prediction of the presence of steatosis among obese children in the present study. Also, according to other studies, no single clinical or laboratory parameters reflect the presence or severity of hepatic fibrosis in children with NAFLD except for significantly different values of BMI (Yang *et al.* 2012). However, looking at the results obtained in the present study, the clear cut trend towards higher increase in activity of AST, ALT, GGT, HA concentration as well as ELF test value has been seen in children with steatosis and showed good agreement with US examination. It is well known, that

finding a good, single marker of early stage of any disease is almost impossible due to inter- and intraindividual variations and preanalytical and analytical factors affecting clinical chemistry measurement.

CONCLUSION

ELF test cannot be used for assessment of steatosis in obese children.

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