Transient Elastography Is a Noninvasive Method to Diagnose Hepatic Fibrosis Stages in Children with Rare Diseases

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The aim of the investigation was to assess diagnostic significance of liver transient elastography (LTE) in children with cystic fibrosis (CF) and mucopolysaccharidoses (MPS) and glycogen storage disease (GSD) to define liver fibrosis stages.

Materials and Methods. We examined 204 children with rare diseases aged from 6 months to 17.5 years, among them there were 141 patients with CF, 25 patients with MPS, and 38 children with GSD. All patients underwent LTE on FibroScan®502 using sensors S+ (with two modes) and M depending on chest circumference.

Results. 42 (29.8%), 7 (28.0%) and 18 (47.4%) patients with CF, MPS and GSD, respectively, had formed fibrotic changes of liver parenchyma of different intensity, and in 12.1, 12.0 and 10.5 cases, respectively, there was marked liver fibrosis or cirrhosis. LTE findings showed pronounced heterogeneity in 40 from 99 (40.4%) CF patients, in 14 from 18 (77.8%) MPS patients, and in 10 from 20 (50.0%) GSD patients, whose median elasticity was consistent with the absence of fibrosis. Some individual measurements demonstrated increased indices (from 5.9 to 75.0 kPa) that can indicate the presence of focal fibrosis or cirrhosis of liver in these patients.

Conclusion. LTE is an informative, noninvasive, safe technique to diagnose various stages of liver fibrosis in children with rare diseases (CF, MPS, GSD), which enables to apply it since the neonatal period. LTE makes it possible to identify diffuse liver fibrosis risk group patients. LTE is efficient for monitoring to assess the liver condition dynamics in children with rare diseases both in outpatient and inpatient investigation period.

Key words: rare diseases; liver fibrosis; transient elastography; METAVIR.

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Recently, there has grown the interest in the study of rare diseases, most of which are caused by genetic mutations. Such diseases are frequently characterized by a chronic progressive course, various organs being involved in the pathological process. In some diseases (cystic fibrosis, mucopolysaccharidoses and glycogen storage disease) liver can be affected.

Cystic fibrosis (CF) is a systemic hereditary disease caused by cystic fibrosis transmembrane regulator (*CFTR*) gene mutation and characterized by the involvement of endocrine glands, severe respiratory and gastrointestinal impairments. Liver involvement revealed in 25–30% CF patients is associated with impaired *CFTR* gene expression in epithelial cells of bile ducts resulting in inspissation of the bile and cholestasia. Due to the occlusion of bile ducts, they dilate, destruct, cholangitis and pericholangitis developing, and further it contributes to portal and periportal fibrosis with altered architectonics of the liver [1–5]. Macrovesicular hepatic steatosis can occur in 28–40% cases. Focality and heterogeneity of parenchymal alterations are specific signs of liver involvement in CF, therefore, the pathology is latent for a long period of time, and clinical manifestations frequently occur in severe hepatic fibrosis or cirrhosis. The incidence of focal biliary cirrhosis is 5–20%. There is information that liver cirrhosis is more common in patients with mutations *G542X*, *W1282X*, *1677delTA*, *delF508* in *CFTR* gene [6–8].

Mucopolysaccharidoses (MPS) are a group of rare lysosomal diseases associated with a genetic defect of enzymatic degradation of carbohydrate branch of glycosaminoglycans accompanied by their deposit in the connective tissue of various organs.

Most of these diseases are inherited by autosomal recessive mode. Depending on an enzyme defect, there have been distinguished up to 11 basic MPS types, e.g.: type I: Hurler syndrome; type II: Hunter syndrome; type III: Sanfilippo disease; type VI: Maroteaux–Lamy syndrome. Clinical conditions manifest themselves during the first three years. Various craniocerebral anomalies are the characteristic features of the pathology: steeple tower head, coarse features with large lips and tongue, low nasal bridge, macrocephaly, short neck, short trunk. There is also the involvement of skeletal system (spinal deformity, chest distortion), joints (stiffness, deformation), internal organs (hepatolienomegaly, cardiac damage), eye alterations (corneal opacity, congestion, and optic atrophy), deafness, changes in muscular tone, muscular hypotrophy, impaired tendon reflexes, growth retardation, mental retardation [9]. In the liver of children with MPS there is excess deposit of glycosaminoglycans, which are hepatotoxic and can result in fibrous foci development [10].

Glycogen storage disease (GSD) is a general term for rare hereditary diseases due to the deficiency of various enzymes participating in glycogen metabolism. Enzyme defects result in abnormalities in their structure and excess glycogen accumulation in body organs and tissues. Depending on the deficiency of a certain enzyme, as well as a type of the tissue affected, currently, there are up to 15 types of the disease distinguished. In particular, the liver and/or muscles undergo pathological changes. Types I, III, VI and IX refer to hepatic forms of GSD [9, 11]. One of the core symptoms of the disease is chronic hypoglycemia aggravating significantly patients' condition, and one of the main clinical sign is marked hepatomegaly due to excess glycogen accumulation in hepatocytes. Low glucose in blood contributes to lipolysis increase, increased concentration of fatty acids in plasma, hyperlipoproteinemia. In addition, increased intensity of triglycerides synthesis and the formation of fat vacuoles in hepatocytes result in hepatic steatosis leading to hepatomegaly. Alongside with progressive dystrophic changes of the liver, inflammatory changes can occur resulting in fibrosis, which frequently leads to cirrhosis [9–13].

No effective pathogenetic therapy for liver disease in CF, MPS and GSD has been developed so far. For hepatoprotective purpose, long-term administration of ursodeoxycholic acid preparations is reasonable. Sclerotherapy or bypass can be used in case of marked portal hypertension, in order to prevent esophageal variceal hemorrhage. Liver transplantation is indicated in end-stage hepatic diseases [14].

In connection with the above mentioned, children with rare diseases require early diagnostics of the liver involvement to assess the disease prognosis and determine the management of such patients. Therefore, there is the demand for an informative technique able to estimate the intensity of structural hepatic changes.

Liver transient elastography (LTE) is one of a promising noninvasive techniques to diagnose a liver fibrosis stage in patients with orphan diseases, LTE being performed using FibroScan®502 (Echosence, France). The principle of the device operation consists in using low-frequency oscillations for quantitative assessment of elasticity as a liver parenchyma state index and the intensity of its fibrotic changes. Unlike ultrasound, LTE has an ultrasonic transducer with a built-in source of mean-amplitude and low-frequency oscillations. The oscillations generated by it are transmitted to hepatic tissues under study and create elastic waves exposing an ultrasonic echo to modulations. The elastic wave velocity depends on hepatic tissue elasticity. The total volume of hepatic tissue under study averaged 6 cm^3 that many times exceeds that used in liver needle biopsy. The results obtained (stated in kilopascals) enable to classify the patients according to the intensity of liver fibrosis, e.g., according to METAVIR scale [15]. Elastography findings are estimated in relation to a median, interquartile range and success rate index measured automatically by the device. Based on the registration study data, in Russia it is assumed that

values ≤5.8 kPa correspond to no fibrosis, while those >12.5 kPa indicate hepatic cirrhosis [16].

The technique has proved to work well both in adults and children, however, most researches concern the study of liver elasticity changes in chronic virus hepatitis B and C [17–22], whereas in children with rare diseases LTE capabilities have been studied insufficiently.

The aim of the investigation was to assess diagnostic significance of liver transient elastography in children with cystic fibrosis, mucopolysaccharidoses and glycogen storage disease to define liver fibrosis stages.

Materials and Methods

Participants. Over a period of 2010–2014, 204 children with rare diseases, aged from 6 months to 17.5 years were examined in Scientific Centre of Children's Health, Ministry of Health of the Russian Federation. Table 1 shows the distribution of children nosologies, age and gender.

The study complies with the declaration of Helsinki (adopted in June, 1964 (Helsinki, Finland) and revised in October, 2000 (Edinburg, Scotland)), and was performed following approval by the Ethic Committee of Scientific Centre of Children's Health, Russian Academy of Sciences (Russia). Written informed consent was obtained from all patients aged 15–17.5 and the parents of those patients who were under 15 in accordance with the Federal Law "The Basic Law on the Health Protection of the citizens of the Russian Federation" dated July, 22, 1993 No.487-1.

Methods. All patients underwent LTE on FibroScan®502 (Echosence, France) using sensors depending on chest circumference (CC): S+ (with two modes) and M. S1 mode with frequency 5 MHz was applied in patients with CC under 45 cm; S2 mode with frequency 5 MHz was used if CC ranged from 45 to 75 cm; M mode with frequency 3.5 MHz was used if CC was over 75 cm. The measuring depth in S1 mode was 15–40 mm away from skin edge; in S2 mode it was 20–50 mm, in M mode: 25–65 mm. The liver area under interest in S1 mode was 2 cm^2, in S2 mode: 2.4 cm^2, and in M mode: 3 cm^2.

Table 1

Distribution of children by the diseases, age and gender

Nosological entity	Number of children	Age, years (Me [25; 75])	Gender	
			Boys	Girls
Cystic fibrosis	141	7.6 [3.4; 13.1]	80	61
Mucopolysaccharidoses	25 (4: with type I, 15: with type II, 4: with type III, 2: with type VI)	6.0 [4.0; 11.0]	21	4
Glycogen storage disease	38 (12: with type I, 11: with type III, 15: with types VI and IX)	8.8 [4.5; 12.0]	25	13

Table 2

Liver elasticity values in different stages of liver fibrosis

Fibrosis stage according to METAVIR	Elasticity value (kPa)
F0	≤5.8
F1	5.9–7.2
F2	7.2–9.5
F3	9.5–12.5
F4	>12.5

LTE was performed in 7–10 areas, a patient being in a supine position, with the patient's right arm on the nape, a sensor mounted in the sixth intercostals space along the right anterior axillary line (liver segment VII view), in the fifth intercaostal space along the right midclavicular line (segment VIII view), in the ninth and tenth intercaostal space along the right midclavicular line (segment VI view), along the median line in epigastric area (the view of segments II, III), in the seventh and eighth intercostals spaces along the right midclavicular line (segment V view), in the fifth intercostals space along the right parasternal line (segment IV view). The study was completed when 10 informative measurements were obtained. The obtained mean value — median — characterized elastic hepatic modulus. The result was expressed in kilopascals, admissible interquartile range (IQR) — not above 1/4 elasticity index. The findings were compared to a liver fibrosis stage determined according to METAVIR scale (Table 2).

Some patients with CF, MPS and GSD according to indications underwent biochemical blood assay and coagulogram as well.

Statistical processing. The data were statistically processed using the application package Statistica 6.0 (StatSoft Inc., USA). We preliminary assessed the distribution nature of values of the variables. For distribution type analysis we used Shapiro–Wilk test and Lilliefors test. The variance of signs was estimated by F-test during ANOVA. Since the distribution of most quantitative indices was not normal, and group variance was not equal, the findings are represented as a median of the parameter, and interquartile range. The differences between the groups were determined using a nonparametric Kruskal–Wallis test. Mann–Whitney test was applied if there were significant differences, and paired comparison was required. The differences were considered significant if $p<0.05$, and highly significant if $p<0.01$.

Results. LTE enabled to receive the data characterizing different liver conditions in children with rare diseases (Table 3). 99 of 141 (70.2%) children with CF had no liver fibrosis, however, the rest 42 (29.8%) were found to have fibrotic changes of various intensity

degrees (median ranged from 5.9 to 48.0 kPa). 18 of 25 (72.0%) children with MPS were found to have no fibrosis, 7 (28.0%) children being revealed fibrotic changes (median ranged from 6.1 to 13.1 kPa). 20 of 38 (52.6%) children with GSD had no fibrosis found, 18 (47.4%) patients were recorded liver parenchyma changes (median ranged from 6.1 to 18.6 kPa).

We calculated median and interquartile ranges of LTE values in patients with CF, MPS and GSD depending on a fibrosis stage according to METAVIR (Table 4). All indices were significant ($p<0.01$).

Some patients with CF, MPS and GSD were indicated to have a biochemical blood assay to determine ALT and AST as the markers of cytolysis syndrome, gamma-glutamyl transpeptidase (GGT) and alkaline phosphatase (ALP) as cholestasia markers in liver involvement. We studied coagulogram indices: prothrombin index (PTI) and international normalized ratio (INR) in order to reveal the signs of protein synthetic liver function abnormality (Table 5).

Table 3

Distribution of patients with rare diseases by liver fibrosis intensity according to transient elastography data

Fibrosis stage according to METAVIR	Number of patients (abs. number/%)		
	Cystic fibrosis (n=141)	Mucopolysaccharidoses (n=25)	Glycogen storage disease (n=38)
F0	99/70.2	18/72.0	20/52.6
F1	14/9.9	2/8.0	11/28.6
F2	11/7.8	2/8.0	3/7.9
F3	7/5.0	2/8.0	—
F4	10/7.1	1/4.0	4/10.5

Table 4

Transient elastography indices (in kPa) in children with rare diseases depending on fibrosis stages according to METAVIR

Fibrosis stage according to METAVIR	Cystic fibrosis		Mucopolysaccharidoses		Glycogen storage disease	
	Me	IQR [25; 75]	Me	IQR [25; 75]	Me	IQR [25; 75]
F0	4.0	3.7; 4.6	4.2	3.7; 4.5	4.2	3.5; 4.7
F1	6.3*	6.0; 6.4	6.6*	6.1; 7.1	6.2*	6.1; 6.5
F2	7.9*	7.6; 8.3	8.7*	8.6; 8.8	8.0*	7.2; 8.8
F3	10.3*	9.8; 10.7	10.5*	10.1; 10.8	—	—
F4	23.6*	14.0; 40.3	—	—	15.5*	12.5; 18.6

* The differences are significant between fibrosis stages within each nosological entity of the disease ($p<0.01$).

On the average, the medians of the laboratory findings under study in patients with CF and MPS were found to range within admissible values. The exception to this rule applied among GSD patients, who appeared to have 2.5 times increased ALT and AST levels and minimal increase in GGT. The comparison of these parameters in patients with no fibrosis and having liver cirrhosis, according to LTE, showed that in cirrhosis ALP and INR levels in blood serum in children with CF were higher, and PTI levels were significantly lower. In addition, there was a tendency for ALT and AST concentration increase in patients with cirrhosis, however, there are no reliably data obtained due to few number of observations. No differences of the indices were found in children with GSD, and among children

Table 5

Laboratory indices in children with rare diseases

Index	Norm	Cystic fibrosis		Mucopolysaccharidoses		Glycogen storage disease	
		Me	IQR [25; 75]	Me	IQR [25; 75]	Me	IQR [25; 75]
ALT (IU/L)	<40	21.0	15.0; 25.0	15.5	13.0; 20.0	96.5	35.0; 200.0
AST (IU/L)	<42	31.0	24.0; 42.0	26.5	22.0; 33.0	110.0	49.0; 209.0
GGT (IU/L)	5–35	11.0	10.0; 16.0	11.0	10.0; 13.0	38.5	13.0; 67.0
ALP (IU/L)	50–450	245.0	197.0; 298.0	160.0	103.0; 200.0	224.5	174.0; 252.0
PTI (%)	70–130	96.0	83.0; 100.0	91.5	85.5; 96.0	110.0	105.0; 118.0
INR	0.85–1.15	1.02	0.98; 1.14	1.05	1.03; 1.1	0.95	0.91; 0.97

N o t e. Number of observations (n) in children with cystic fibrosis: ALT — 41, AST — 41, GGT — 41, ALP — 41, PTI — 28, INR — 28; in children with mucopolysaccharidoses: ALT — 18, AST — 18, GGT — 11, ALP — 11, PTI — 11, INR — 11; in children with glycogen storage disease: ALT — 38, AST — 38, GGT — 38, ALP — 38, PTI — 38, INR — 38.

Table 6

Laboratory findings in children with rare diseases without liver involvement and those with cirrhosis according to transient elastography (Me [25; 75])

Index	Cystic fibrosis		Glycogen storage disease	
	F0	F4	F0	F4
ALT (IU/L)	17.5 [15; 23]	31.5 [19; 56]	98.0 [48.5; 217.5]	123.0 [21.0; 135.0]
AST (IU/L)	30.0 [24; 34]	60.0 [30; 61]	110.0 [43.0; 218.5]	156.0 [24.0; 221.0]
GGT (IU/L)	12 [9; 16]	15 [11; 18]	36.0 [12.5; 59.0]	77.0 [9.0; 320.0]
ALP (IU/L)	243.0 [186; 269]	349 [323; 410]*	215.0 [168.0; 235.5]	258.0 [223.0; 265.0]
PTI (%)	98 [86; 104]	77.5 [72.5; 88.5]*	110.0 [107.0; 114.0]	118.0 [110.0; 127.0]
INR	1.0 [0.98; 1.1]	1.19 [1.09; 1.24]*	0.95 [0.92; 0.97]	0.91 [0.87; 0.95]

* Significant differences between cystic fibrosis children with F0 and F4 stages according to METAVIR ($p<0.01$).

Table 7

Relations of transient elastography indices with some laboratory indices in children with cystic fibrosis

Parameters	r (according to Spearman)	p
LTE and ALT	0.46	0.002
LTE and AST	0.4	0.009
LTE and GGT	0.69	0.019
LTE and PTI	–0.5	0.039
LTE and INR	0.55	0.018

with MPS no comparison was made due to the fact that only 1 patient had liver cirrhosis (Table 6).

Correlation analysis revealed the relations of LTE indices with some laboratory findings characterizing liver involvement in patients with CF (Table 7). No similar relations were found in children with MPS and GSD.

It is significant that in 40 of 99 (40.4%) children with CF, whose elasticity median corresponded to no fibrosis (F0), LTE findings were characterized by marked heterogeneity: in certain measurements they were recorded to have increased indices (from 5.9 to 75.0 kPa). Children with MPS and GSD had similar findings: 14 of 18 (77.8%) patients with MPS with median corresponding to F0 stage appeared to have some foci with increased values ranging from 5.9 to 75.0 kPa, and 10 of 20 (50.0%) patients with GSD, median F0, were revealed to have some foci with increased values ranging from 6.3 to 69.1 kPa.

Discussion. Scleral subicterus, ochrodermia, palmar erythema, telangiectasia, anterior abdominal wall varices are known to be general clinical signs of liver diseases in any pathology, and in particular, in orphan diseases. There can also be palpated enlarged, indurated liver, in cirrhosis it has “stone-like” structure, hummocky surface, irregular and sometimes tender edge combined with splenomegaly. Among laboratory changes there were increased levels of ALT and ASR, GGT and ALP in blood serum. In case of impaired protein-synthetic function, there can be decreased PTI and increased INR [23].

Ultrasound is used as a screening liver imaging technique, which can help detect hepatomegaly, liver parenchymal changes in micro- or medium-sized focal diffuse heterogeneity and hyperechogenicity. In case of cirrhotic transformation, irregular liver edge combined with diffusely heterogeneous coarse parenchyma is seen. Color flow mapping shows the signs of portal hypertension. However, in certain cases due to heterogeneous hepatic changes in CF and steatosis in GSD revealed histologically, ultrasonic data can be less informative to have a true picture of hepatic structure, particularly, at early stages. Abdominal computed tomography and magnetic resonance imaging are more indicative in liver cirrhosis. Needle biopsy followed by a morphological study of a tissue sample is a gold standard in any liver disease diagnosis. However, it is invasive, complication risky, and has some contraindications; for this reason it is not frequently used in children with rare diseases characterized by an unstable course with decompensation periods [24].

In this regard, LTE application in children suffering from rare diseases accompanied by liver diseases holds much promise. The advantages of the method are the following: non-invasiveness, high reproducibility, examination rapidity and simplicity (an examination takes 3–5 min), the measurement is performed just in liver tissue view (those performed in other organ views are considered to be incorrect, and not registered), the tissue volume under study is 100–200 times as large compared to that used in liver biopsy.

Our findings suggest that fibrotic parenchymal changes of various intensity, according to METAVIR, develop in 29.8, 28.0 and 47.4% children with CF, MPS and GSD, respectively, and 12.1, 12.0 and 10.5% cases have marked liver fibrosis or cirrhosis. Generally, our data are consistent with few foreign research reports.

So, Witters et al. [25] examined 66 children with CF, their mean age being 13.6±7.8 years. 14 patients (21%) among them were recorded to have increased LTE indices, which significantly grew in children with clinical signs of liver involvement: 11.07±5.51 versus 5.08±3.45 kPa in control (p<0.0001); with laboratory findings: 7.4±3.1 versus 5.42±4.08 kPa (p=0.013), and sonographic signs of hepatic pathology: 8.19±5.96 versus 4.27±0.94 kPa (p<0.0001). Three patients who had maximum LTE values (13.6, 20.5 and 29.1 kPa) suffered from esophageal varicose veins dilatation (EVVD). One patient (LTE=11.6 kPa) underwent biopsy, the morphology reveling periportal and porto-portal fibrosis.

Malburnot-Wagner et al. [26] examined 18 children with CF aged from 9 to 18 years. Their LTE indices ranged from 3 to 75 kPa, a median being 16.4 [12.2, 23.7] kPa. In addition, LTE values were significantly higher in children with EVVD compared to those without EVVD: 22.4 [14.4, 30.4] versus 7.9 [4.4, 13.7] kPa (p=0.01). Minimal median value in children with EVVD was 12 kPa, and maximal value in case of EVVD absence was 17.5 kPa. LTE indices had no significant difference in children without EVVD and with stage I EVVD compared to patients with stage II–III EVVD.

Thus, the relation of LTE indices with clinical laboratory and instrumental data characterizing liver involvement proves the technique to be informative when determining liver fibrosis stage in children.

It is interesting to note that some examined children with normal liver elasticity median had increased LTE indices in certain parts. Patients with CF should be paid particular attention. Based on world literature data on typical intense heterogeneity of structural liver changes in the disease [6–8], we can suspect the presence of focal sclerotic hepatic changes in such patients and refer them to diffuse liver parenchyma risk group.

We found no data on LTE application in children with MPS and GSD in the literature available, so our study is actually the first attempt to highlight the problem. Local increase of some LTE values in MPS is probably to be related to excess deposit of glycosaminoglycans, and in GSD: glycogen accumulation in liver parenchyma.

Conclusion. Liver transient elastography is a promising and effective method to diagnose different fibrosis stages in children with rare diseases (cystic fibrosis, mucopolysaccharidoses, glycogen storage disease), which can be used since neonatality. The technique, informative, noninvasive and safe, enables to diagnose diffuse liver fibrosis risk group patients. Transient elastography application is reasonable for monitoring to assess liver state dynamics in children with rare diseases both during outpatient and inpatient examinations.

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