ADVANTAGES OF BIOCHEMICAL METHODS OF DIAGNOSING FIBROSIS IN NON-ALCOHOLIC FATTY LIVER DISEASE IN ADOLESCENTS WITH OBESITY

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Abstract: Non-alcoholic fatty liver disease occurs in most obese people, the main pathway of which is the process of fibrogenesis. This disorder is currently classified into two types: hepatic steatosis and nonalcoholic steatohepatitis. Hepatic steatosis is a reversible condition in which large vacuoles of triglyceride fat accumulate in the liver cells, causing nonspecific inflammation. Most people with this condition experience few, if any, symptoms, and it does not usually lead to scarring or serious liver damage. The majority of patients with nonalcoholic fatty liver disease have this type. Nonalcoholic steatohepatitis is the more severe, progressive form that involves not only fat accumulation (steatosis) in the liver but also inflammation. Steatohepatitis can lead to fibrosis and eventually to cirrhosis, which is severe scarring that can lead to liver failure. The real frequency of the prevalence of the disease is difficult to establish, due to the insufficient use of non-invasive screening diagnostic methods, through which it is possible to detect the initial forms of the disease.

The aim: to study the diagnostic significance of the serum biomarkers of liver fibrogenesis in adolescents with obesity.

Methods. On the base of the Department of Endocrinology, SI "Institute of children and adolescence health care of NAMS" (Kharkov) 226 patients with obesity aged 8–18 years were examined. Investigation of liver fibrosis consisted of measurement in blood the levels of fibronectin, collagen type IV, N-terminal propeptides and C-terminal telopeptides of type I collagen by IFA method.

Results. The study of liver fibrogenesis revealed a significant increase in levels of type IV collagen and fibronectin in children with obesity ($p<0.05$). As diagnostic criteria for two physiologically diverse processes – fibrogenesis and fibrolysis, the levels of N-terminal propeptides and C-terminal telopeptides of type I collagen, respectively, were determined. The serum level of N-terminal propeptides of type I collagen significantly exceeds the normal values in all children with obesity, in contrast to the children of the control group ($p<0.05$).

Conclusion. It has been established that a biochemical method for determining the level of type IV collagen, fibronectin, N-terminal propeptides and C-terminal telopeptides of type I collagen has a high sensitivity for the diagnosis of liver fibrogenesis.

Keywords: adolescents, non-alcoholic fatty liver disease, liver fibrosis, obesity, diagnostics methods.

1. Introduction

Data on present problem published in the last years in scientific literature testify interlinking of metabolic syndrome to typical gastroenterological manifestations – steatosis of the liver and pancreas, cholelithiasis, cholesterosis of the gallbladder [1, 2]. The basic role in development of metabolic disorders with insulin resistance syndrome is supposed, on one hand, to steatosis of the liver and, on the other hand, to hyperinsulinemia, hyperglycemia and dyslipidemia, that aggravate gastroenterological problems [3, 4]. In the author's opinion, inflammation, intimately conjugated to development of steatohepatitis, concomitant diseases and their complications (mainly obesity, which is obligatory for metabolic syndrome) is the trigger of comorbid digestive pathology progression at metabolic syndrome [5, 6].

A healthy liver can readily handle a lifetime's worth of dietary fat, but a failing liver cannot. The liver can be overtaxed by excess dietary fat. Triglycerides can build up in hepatocytes if the liver's mitochondrial beta-oxidation and very–low-density lipoprotein production are insufficient to handle the fatty acid load. Over time, this fat accumulation can lead to scarring, inflammation, fibrosis, and cirrhosis—the progressive stages of nonalcoholic fatty liver disease (NAFLD) [7].

Currently, nonalcoholic fatty liver disease is one of the most common chronic liver diseases in the world [8]. NAFLD is classified into two types: hepatic steatosis and nonalcoholic steatohepatitis (NASH). Hepatic steatosis is a reversible condition in which large vacuoles of triglyceride fat accumulate in the liver cells, causing nonspecific inflammation. Most people with this condition experience few, if any, symptoms, and it does not usually lead to scarring or serious liver damage. The majority of patients with NAFLD have this type. NASH is the more severe, progressive form that involves not only fat accumulation (steatosis) in the liver but also inflammation. Steatohepatitis can lead to fibrosis and eventually to cirrhosis, which is severe scarring that can lead to liver failure [9, 10].

NAFLD occurs in most people with obesity, the main path of progression is the process of fibrogenesis [11, 12], which is accompanied by the deposition of components of the extracellular matrix (collagen of different types, fibronectin, etc.) in perisinusoidal spaces, which leads to structural and functional failure of the organ.

The real frequency of the prevalence of the disease is difficult to establish, due to the insufficient use of non-invasive screening diagnostic methods, through which it is possible to detect the initial forms of the disease [13]. Studies confirm the possibility of diagnosing fibrosis in non-alcoholic fatty liver disease using non-invasive methods [14, 15], but their use in pediatric practice is not known until now. Therefore, the search for biochemical markers of liver fibrosis, which are highly sensitive, informative and can be used in children, is very relevant today.

The aim of our research. To improve the effectiveness of noninvasive diagnosis of liver fibrosis in adolescents with obesity using serum biomarkers of liver fibrogenesis.

2. Methods

On the base of SI "Institute of children and adolescents health care of the NAMS" were inspected 226 patients with obesity in age 8–18 years and 30 healthy children for control group.

Investigation of liver fibrogenesis consisted of measurement in blood serum of fibronectin level («Biochimmak» (Russia), 70±14.0 mkg/ml), serum collagen type IV («Argutus Medical» (Japan), 99±2,3 mkg/l), N-terminal propeptides of type I collagen (N-TP) («Biomedica» (Austria)). C-terminal telopeptides of type I collagen (C-TT) («Immunodiagnostic Systems Ltd» (UK)) is index of fibrolysis. Statistical processing was made by program Statistics+.

The normal content of propeptides and telopeptides according to the referential values of the test kit tested on the control group, are presented in the **Table 1**.

Table 1

The content of normal level N-terminal propeptides and C-terminal telopeptides of type I collagen in serum in children of different age groups

N-terminal propeptides of type I collagen, pmol/l					
Prepubertal period		Pubertal period		Postpubertal period	
boys	girls	boys	girls	boys	girls
4.52±0.324	5.87±0.334	6.1±0.274	4.98±0.268	4.9±0.352	3.75±0.147
C-terminal telopeptides of type I collagen, ng/ml					
1.883±0.374	2.029±0.361	2.281±0.474	2.266±0.368	1.069±0.552	0.821±0.447

3. Results

It was found that 113 (50.0±3.33 %) patients had insulin resistance (IR). The study of liver fibrogenesis revealed a significant increase in levels of type IV collagen and fibronectin in children with obesity (p<0.05), (**Table 2**). The levels of fibronectin blood significantly differed in groups, depending on the presence of IR, which apparently indicates a more severe liver damage in children with IR (p<0.05).

Table 2

Levels of collagen type IV and fibronectin in adolescents with obesity, depending on the presence of IR (M±σ)

Children with obesity	n	Collagen type IV, mkg/l	Fibronectin, mkg/ml
IR +	113	107.61±7.04*	115.86±7.20*, **
IR –	113	103.76±8.31*	93.00±6.31*
Control group	30	85.91±2.38	78.36±2.12

*Note: * – Difference between patients with obesity and healthy children (p<0.05); ** – Difference between patients with IR and without it (p<0.05)*

As diagnostic criteria for two physiologically diverse processes - fibrogenesis and fibrolysis, the levels of N-TP and C-TT of type I collagen, respectively, were determined. The serum level of N-TP of type I collagen significantly exceeds the normal values in all children with obesity, in contrast to the children of the control group (p<0.05) (**Table 3**).

In patients with IR, the level of N-TP of type I collagen were more elevated than in the group without IR, which indicates a more intensive process of liver fibrogenesis in the presence of insulin resistance.

The levels of C-TT of type I collagen in children with obesity were within the norms and did not differ statistically significantly from those in the control group (p>0.05) (**Table 4**).

Exceptions are children of early pubertal age, in whom the indicators of the marker of fibrolysis were significantly lower than in children of the control group (p<0.05). Apparently, this is due to the predominance of fibrogenesis processes over fibrolysis, which is typical for liver fibrosis.

4. Discussion

The obtained results do not contradict the existing ones and confirm the expediency of using non-invasive methods of diagnosing non-alcoholic fatty liver disease in pediatric practice. The advantage of using these methods is to improve the early diagnosis of non-alcoholic fatty liver disease in children, the observation and prevention of progression and complications. Although liver biopsy is the most accurate modality to diagnose and stage the severity of NASH, this method suffers from being invasive, costly, associated with potential complications, and plagued with interobserver variability of individual pathological features. Of the various serum markers, fibronectin, collagen type IV, N-terminal propeptides and C-terminal telopeptides of type I collagen seems to best predict fatty liver disease, the NAFLD Fibrosis Score is most closely correlated with fibrosis, and transient elastography can be used for diagnosis of cirrhosis, or to exclude cirrhosis, although its utility is limited by obesity [16].

Thus, non-invasive diagnostic methods using serum biomarkers of hepatic fibrosis (type IV collagen, fibronectin, N-terminal propeptides and C-terminal telopeptides of type I collagen) have confirmed their diagnostic sensitivity in establishing the presence of liver fibrogenesis processes on the early stages formation in children with obesity. In the future, this will increase the efficiency of early diagnosis of fatty liver disease, dynamic observation of a patient with control of biochemical parameters of liver fibrosis and improve treatment and prevention measures.

Table 3

The levels of N-terminal propeptides of type I collagen in children with obesity (M±σ)

Children with obesity	N-terminal propeptides of type I collagen, pmol/l					
	Prepubertal period (n=32)		Pubertal period (n=92)		Postpubertal period (n=102)	
	boys	girls	boys	girls	boys	girls
IR + (n=113)	9.03±0.21*, **	11.12±1.60*, **	8.6±1.04*	7.42±0.76*	8.28±0.80*, **	5.51±0.88*, **
IR – (n=113)	6.98±0.65*	5.9±0.77*	9.536±1.84*	6.903±0.61*	7.806±0.94*	4.536±0.52*
Control group (n=30)	4.45±0.13	5.64±0.14	6.21±0.16	4.81±0.12	5.08±0.13	3.79±0.11

*Note: * – Difference between patients with obesity and healthy children (p<0.05); ** – Difference between patients with IR and without it (p<0.05)*

Table 4

The levels of C-terminal telopeptides of type I collagen in children with obesity (M±σ)

Children with obesity	C-terminal telopeptides of type I collagen, ng/ml					
	Prepubertal period (n=32)		Pubertal period (n=92)		Postpubertal period (n=102)	
	boys	girls	boys	girls	boys	girls
IR + (n=113)	1.86±0.41	1.921±0.14	1.88±0.18*	1.673±0.18*	1.291±0.08	1.134±0.14
IR – (n=113)	1.72±0.25	1.927±0.42	1.888±0.34*	1.281±0.10*	1.218±0.13	0.733±0.06
Control group (n=30)	1.52±0.32	1.89±0.43	2.61±0.71	2.47±0.68	1.23±0.25	0.89±0.19

*Note: * – Difference between patients with obesity and healthy children (p<0.05)*

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