MORPHOFUNCTIONAL CHANGES OF GALLBLADDER AND BIOCHEMICAL PARAMETERS OF LIPID METABOLISM IN CHILDREN WITH LIVER STEATOSIS

Abstract. Background. The aim of study was to investigate peculiarities of structural and functional disorders of gallbladder in children with non-alcoholic fatty liver disease. Materials and methods. The study was conducted in 51 children aged 10–17 years. Patients were divided into four groups: the 1st group consisted of 11 children with biliary normokinesia without steatosis; the 2nd group — 20 children with biliary hypokinesia without steatosis; the 3rd group — 11 children with biliary normokinesia and steatosis, and the 4th group — 9 children with biliary hypokinesia and steatosis. Anthropometric parameters were measured: height, weight, abdominal circumference, body mass index. Functional state of gallbladder was investigated with the help of ultrasound. Such biochemical parameters, as total cholesterol, triacylglycerols, levels of high-density lipoprotein cholesterol, low-density lipoprotein cholesterol and very-low-density lipoprotein cholesterol were defined, atherogenic index was calculated according to Friedewald formula. Results. Present study shows overweight (in 15 % of patients), obesity (in 85 % of patients), increase in the gallbladder volume by 30–50 % (p < 0.05), in the density of gallbladder wall by 12–16 % (p < 0.05) and its echogenicity by 24 % (p < 0.05) in children with steatosis versus group without steatosis and gallbladder normokinesia. Positive correlation between wall thickness and steatosis has been established. Thus, weight increase may potentially play a role in lipid disorders and biliary dysfunction. The 70 % of children with obesity had pathological changes of lipid levels, such as 74 % — increased blood atherogenicity. Conclusions. Physicians must necessarily correct a functional state of gallbladder during the treatment of obesity in children and adolescents. Only comprehensive approach to the treatment allows prevention of non-alcoholic fatty liver disease and its complications. Keywords: biliary dysfunction; steatosis; obesity; dyslipidemia

Introduction

Since 1980, number of patients with obesity increased twice. In 2014 more than 41 million of children older than 5 years had overweight. Physicians set obesity apart as a systemic disease which is based on lipid and carbohydrate metabolism disorders [1]. In blood of patients with overweight level of free fatty acids is increased. It leads to fat accumulation in internal organs and visceral obesity. Therefore, overweight is a main etiological factor in the pathogenesis of metabolic disorders, such as non-alcoholic fatty liver disease (NAFLD) and functional gallbladder disorders (FGD) [2–4]. In Calasani’s study (2012) among adult patients with NAFLD 90% had increased body mass index (BMI) and visceral obesity, in 69 % of cases diabetes mellitus type 2 was diagnosed, in 50 % of cases — high level triacyl glycerides (TG) and low level of high density lipoprotein (HDL) in blood serum were found [5]. Such changes are common for pediatric patients. Clinical investigation showed NAFLD in one third of children with obesity, which was often accompanied by dyslipidemia, intolerance to carbohydrates and insulin resistance [6]. Etiology, development and diagnosis of steatosis in children are specific. The youngest age of steatosis diagnosis is 2 years [7]. Ecological and genetic factors play important role in NAFLD development [5].
Our previous investigations demonstrated that more than 50% of children with NAFLD had FGD [8]. The previous studies showed positive correlation between total obesity, fatty liver and fatty gallbladder in adults. Under these conditions the functional state of liver and gallbladder is low. The existing literature data describe a direct link between the overall adult obesity, fatty liver and fatty gallbladder, thus decreasing their functional ability. Unfortunately to date there is no detailed analysis of the relationship of these abnormalities in pediatric patients.

**Aim of study** — to investigate peculiarities of structural and functional disorders of gallbladder among children with non-alcoholic fatty liver disease.

**Materials and methods**

Study was conducted in children and adolescents searching for medical help with gastro-intestinal tract disorders in SD «Institute of Gastroenterology of the National Academy of Medicine Sciences of Ukraine». All patients had given their agreement to participation in the study.

The study was conducted in 51 children aged 10–17 years. Patients were divided into four groups: the 1st group consisted of 11 children with biliary normokinesia without steatosis; the 2nd group — 20 children with biliary hypokinesia without steatosis; 3rd group — 11 children with biliary normokinesia and steatosis, and the 4th group — 9 children with biliary hypokinesia and steatosis.

Anthropometric parameters were measured: height, weight, waist circumference, body mass index:

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BMI = \frac{\text{body mass, kg}}{\text{height, m}^2} \quad [9].
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Thickness of gallbladder wall and its volume were measured in all patients with ultrasound (SH-2000 Honda Electronics) in real time scale, on an empty stomach. Analysis of changes in abdominal organs included size measurement, profile, acoustic structure and echogenicity by generally accepted methods [10].

We had studied gallbladder function with ultrasound on the background of choleretic breakfast. As choleretic breakfast we used more physiological routine food loading: 40 g of wheat bread, 20–25 g of butter, 150–200 ml black tea with 5 g of sugar [11].

Gallbladder volume was measured: with empty stomach and after breakfast (5, 10, 45 and 60th minutes).

Decreasing of gallbladder volume on 45th or 60th minutes after breakfast for 34–64% was the evidence of gallbladder normokinesia, less than 33% — hypokinesia [11].

Diagnosis of liver steatosis was realized by measurement of the controlled attenuation parameter (CAP) with FibroScan®502 Touch F60156 (Echosens, France). This parameter was correlated with the degree of fatty liver. Its level higher than 232 db/m² confirmed that total amount of fatty hepatocytes was more than 10%.

Such biochemical parameters as total cholesterol (CH), triacylglicerols (TG), phospholipids (PL), levels of high density lipoprotein-cholesterol (HDL), low density lipoprotein-cholesterol (LDL) and very low density lipoprotein-cholesterol (VLDL), atherogenic coefficient (AC) were analysed with Cormey test-kits (Poland) and biochemical analyzer Stat Fax 1904 Plus, Awareness Technology (USA) calculated according to Friedewald formula.

Data of NCEP (American National Cholesterol Education Program) and NHANES (The National Health and Nutrition Examination Survey, USA) were used as lipid metabolism reference criteria in children. The normal CH levels for 1–19-year-old children were 2,96–4,4 mmol/l, TG — 0,40–0,9 mmol/l, HDL — 0,99–1,59 mmol/l, LDL — 1,63–2,59 mmol/l and VLDL — 0,22–0,40 mmol/l.

| Parameters | Group 1st, normokinesia | Group 2nd, hypokinesia | Group 3rd, normokinesia | Group 4th, hypokinesia |
|------------|-------------------------|------------------------|-------------------------|------------------------|
| Age, years | 9.80 ± 1.21 | 11.91 ± 0.69 | 12.33 ± 0.92 | 13.29 ± 1.08 |
| Height, cm | 145.50 ± 6.90 | 158.45 ± 2.43 | 165.42 ± 5.46* | 161.14 ± 4.89 |
| Weight, kg | 44.55 ± 3.49 | 54.84 ± 1.93 | 83.17 ± 5.47* | 75.03 ± 7.69* |
| Waist circumference, cm | 65.83 ± 1.45 | 76.27 ± 8.34 | 99.17 ± 4.03* | 85.86 ± 2.90* |
| Body mass index, kg/m² | 21.30 ± 0.82 | 22.37 ± 0.68 | 30.43 ± 1.04** | 27.43 ± 1.75* |
| Subcutaneous fat, mm | 18.10 ± 0.11 | 26.11 ± 2.05 | 24.94 ± 2.61* | 29.05 ± 2.15* |
| Epigastric fat, mm | 6.50 ± 0.10 | 4.86 ± 0.28 | 8.20 ± 1.50* | 7.02 ± 0.40 |
| Intraperitoneal fat I, mm | 38.30 ± 2.13 | 29.22 ± 3.26 | 47.21 ± 4.41 | 48.60 ± 2.30* |
| Intraperitoneal fat II, mm | 49.40 ± 2.40 | 40.89 ± 3.64 | 58.50 ± 4.37 | 61.15 ± 4.05* |
| Intraperitoneal fat III, mm | 17.40 ± 0.38 | 18.06 ± 1.69 | 22.41 ± 2.48* | 20.45 ± 5.15 |
| Fatty index | 0.35 ± 0.08 | 0.44 ± 0.11 | 0.41 ± 0.05 | 0.88 ± 0.16** |

Notes: * — p < 0.05; ** — p < 0.01 — significance of differences between parameters in compare with group I.
Data from all groups were compared using the Student unpaired t-test by SPSS 9.0 for Windows. Difference average values were considered as probable in p < 0,05 and p < 0,01 [13].

Results
Study showed no difference in patients age and height between groups (table 1), but body weight was significantly increased in groups 3rd and 4th on average 45 % in comparison with 1st group.

High body weight led to BMI increasing by 26 % in children with steatosis in comparison with children without steatosis. Abdominal type of obesity was presented in children with overweight. In 3rd group waist circumference increasing in 1,8 times was observed, subcutaneous fat increasing — by 59 % and intraperitoneal fat increasing — by 10–57 %. So, patients from groups 3rd and 4th were characterized by overweight (15 % of cases) and obesity (85 % of cases) (p ≤ 0,05). In 1st and 2nd groups obesity was observed in 43 and 24 % respectively.

Increasing of gallbladder volume by 30–50 % (p < 0,05), density of gallbladder wall by 12–16 % (p < 0,05) and its echogenicity by 24 % (p < 0,05) in children with steatosis was observed in comparison with group without steatosis and normokinesia of gallbladder (table 2).

Positive correlation between wall thickness and steatosis has been established: r = 0,39 (t = 2,76; p = 0,03) and r = 0,35 (t = 2,4; p = 0,01). Thus, weight increase may potentially play a role in lipid disorders and biliary dysfunction. It was confirmed with 63 % of gallbladder hypokinesia in children with obesity [6].

Decreasing of gallbladder motility directly influenced on digestion and absorption of lipids in the intestine, such as total lipid metabolism in organism [2]. About 70 % of children with obesity had a pathological changes of lipid levels, such as 74 % — increasing of blood atherogenicity.

In our study hypercholesterinemia was observed in every group, its frequency was 50 % higher among patients with gallbladder hypokinesia: 60 % patients in 1st group and 29 % patients in 4th respectively (fig. 1).

Thus, hypertriacylglycerolemia prevailed among patients with steatosis (fig. 2). It could be related to high triacylglycerides production in the liver as a main mechanism of steatosis development.

Analysis of the lipid fraction ratio in the blood serum depending on gallbladder functional state and liver steatosis in children showed changes in TG, PL, HDL, LDL, VLDL, CH/PL levels and AC. The mild atherogenic dyslipidemia was found in the 3rd group. There were increasing in 1,4 times TG level (p < 0,05), VLDL — in 1,5 times (p < 0,05) and increasing of CH/PL rate in 1,3 times (p < 0,05), in comparison with 1st group (table 3).

Dyslipidemia in patients from 4th group was combined with decreased PL level in blood serum in 1,5 times (p < 0,05), HDL — in 1,5 times (p < 0,05) and CH/PL ratio increasing in 3,6 times (p < 0,05) compared with 1st group.

Discussion
Low gallbladder motility with increased volume and wall thickness were found in children with steatosis. This disorder was accompanied by atherogenic changes in lipid fractions (high TG and VLDL levels). They were more severe in groups with steatosis. So, obesity and overweight are underlying causes in NAFLD development, and FGD leads to the complication of disease. It also does not exclude the deterioration functional status of the gallbladder due to general obesity.

Our data demonstrated an increasing of gallbladder volume and wall thickness in children with steatosis. Significant increase of these parameters in III and IV groups in comparison with I group was showed. Also, motility of gallbladder in these groups was less than in children without steatosis.

**Figure 1. Percent of patients with hypercholesterinemia in groups**

**Figure 2. Percent of patients with hypertriacylglycerolemia in groups**

| Parameters       | S(–) | S(+) |
|------------------|------|------|
| **Group 1st, normokinesia** |      |      |
| Group 2nd, hypokinesia |      |      |
| n = 11            |      |      |
| Volume, cm³       | 16.64 ± 1.61 | 20.44 ± 2.14 |
| Wall thickness, mm | 1.89 ± 0.10  | 1.87 ± 0.16  |
| Increased wall echogenicity, % | 27   | 30   |
| **Group 3rd, normokinesia** |      |      |
| Group 4th, hypokinesia |      |      |
| n = 9             |      |      |
| Volume, cm³       | 21.77 ± 2.97* | 24.80 ± 3.88* |
| Wall thickness, mm | 2.15 ± 0.19*  | 2.24 ± 0.23*  |
| Increased wall echogenicity, % | 36   | 45*  |

Notes: * — p < 0,05.
Correlation between NAFLD and FGD can be explained by lipotoxicity effect which leads to lipid accumulation outside of adipose tissue. Free fatty acids and cholesterol play the key role in this mechanism [14]. It is assumed, that cholesterol increase and relation of cholesterol to phospholipids is a cause of low gallbladder motility because of humoral response inhibition by neuronal transmitters such as acetylcholine, cholecystokinin and neuronal peptide Y [15–17]. It was evidenced in the experiments with animals. Blocking of intestinal absorption of cholesterol led to decrease of lipids level in gallbladder wall and improved gallbladder motility [18].

Other eventual mechanism of low gallbladder motility in NAFLD is an effect of hyperproduction of cytokines in adipocytes which leads to visceral organ inflammation. Numerous studies showed the fact that adipocytes is capable to produce inflammatory molecules, including TNF-α, IL-6 and cause inflammation in other organs [19–21].

So, inflammation in patients with overweight and obesity cause weak function of gallbladder wall and decrease absorption and secretion ability [22, 23]. Our results proved this conception because patients with NAFLD in our research groups often had a high BMI and enlarged gallbladder volume.

Overweight is one of the causes of gallbladder hypokinesia. We suggest that two mechanisms may play role in development of gallbladder hypokinesia in steatosis. They are based on accumulation of fat in gallbladder wall and decreasing of humoral response to neurotransmitters action as a result of cholesterol/phospholipids relation disruption and inflammation. In our opinion, physicians must necessarily correct a functional state of gallbladder during treatment of obesity in children and adolescent. Only complex approach of treatment allows prevention of non-alcoholic fatty liver disease and its complications [24].

| Parameters  | Group 1st, normokinesia S(–) | Group 2nd hypokinesia S(+) | Group 3rd, normokinesia S(–) | Group 4th hypokinesia S(+) |
|------------|-----------------------------|--------------------------|-----------------------------|--------------------------|
| CH, mmol/l | 4.28 ± 0.20                 | 4.50 ± 0.30              | 3.99 ± 0.24                 | 4.04 ± 0.24              |
| TG, mmol/l | 0.49 ± 0.11                 | 0.70 ± 0.11*             | 0.72 ± 0.12*                | 0.93 ± 0.15*             |
| PL, mmol/l | 2.79 ± 0.20                 | 2.41 ± 0.35              | 2.39 ± 0.26*                | 1.95 ± 0.46*             |
| HDL, mmol/l| 1.08 ± 0.09                 | 0.97 ± 0.06              | 0.95 ± 0.08                 | 0.74 ± 0.12**            |
| LDL, mmol/l| 3.02 ± 0.18                 | 2.85 ± 0.19              | 2.71 ± 0.22                 | 2.79 ± 0.23              |
| VLDL, mmol/l| 0.22 ± 0.48                | 0.32 ± 0.05*             | 0.33 ± 0.05**               | 0.43 ± 0.07*             |
| CH/PL      | 1.65 ± 0.19                 | 1.81 ± 0.22              | 2.04 ± 0.05*                | 5.98 ± 0.45**            |
| AC         | 3.12 ± 0.30                 | 3.70 ± 0.39*             | 3.54 ± 0.54*                | 4.25 ± 0.53**            |

Notes: * — p < 0.05; ** — p < 0.01 — significance of differences between parameters in compare with group I.

Conclusions

1. Overweight and abdominal type of obesity play key role in the development of fatty liver in children.
2. Decreased gallbladder contractility lead to dislipidemia, such as hypercholesterinemia development whereas as steatisis is associated with hypertriglyceridemia.
3. Hepatic steatosis formation is associated with gallbladder volume elevation, gallbladder wall thickness and echogenicity increasing, gallbladder motility impairment probably due to fat accumulation and cholesterol/phospholipids relation disruption.
4. We recommend an obligatory functional gallbladder state correction in children with fatty liver.

Conflict of interests. Authors declare the absence of conflict of interests.

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методы. Под наблюдением находился 51 пациент в возрасте от 10 до 17 лет, которые по данным ультразвукового обследования моторно-эвакуаторной функции желчного пузыря и транзиентной эластографии печени были разделены на 4 группы: I — 11 детей с нормокинезией желчного пузыря без стеатоза печени, которую выбрали в качестве группы контроля; II — 20 детей с гипокинезией желчного пузыря без стеатоза печени; III — 11 детей с нормокинезией желчного пузыря и стеатозом печени и IV — 9 детей с гипокинезией желчного пузыря со стеатозом печени. Оценивали антропометрические данные: рост, вес, окружность талии, индекс массы тела. Функциональное состояние желчного пузыря определяли с помощью ультразвуковой диагностики. В качестве исследуемых маркеров липидного спектра сыворотки крови использовали содержание общего холестерина, триглицеридов, липопротеидов высокой и низкой плотности, липопротеидов очень низкой плотности и рассчитывали коэффициент атерогенности. 

Результаты. Установлено, что для пациентов со стеатозом характерными были лишний вес (у 15 % пациентов), ожирение (у 85 % пациентов), увеличение объема желчного пузыря на 30–50 % (р < 0,05), плотности стенок — на 12–16 % (р < 0,05) и эхогенности стенки — на 24 % (р < 0,05) в сравнении с группой без стеатоза и с нормокинезией желчного пузыря. Выявлена положительная связь между толщиной стенки и наличием стеатоза. Таким образом, избыток веса может быть одним из факторов развития нарушений липидного обмена, а также билиарной дисфункции. Выявлено, что у 70 % детей с ожирением имеются в наличии патологические изменения уровня липидов, в том числе у 74 % детей — увеличение атерогенных свойств крови. 

Выводы. Назначая лечение при ожирении детей и подростков, следует обязательно осуществлять коррекцию функционального состояния желчного пузыря. Только комплексный подход к лечению даёт возможность предотвратить развитие осложнений неалкогольной жировой болезни печени.

Ключевые слова: билиарная дисфункция; стеатоз печени; ожирение; дислипидемия