© Pediatr Med Rodz 2019, 15 (3), p. 252–257
DOI: 10.15557/PiMR.2019.0042

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Received: 07.01.2019
Accepted: 12.02.2019
Published: 29.11.2019

Non-alcoholic fatty liver disease in children: pathogenesis and diagnostic and therapeutic possibilities

Niealkoholowa stłuszczeniowa choroba wątroby u dzieci – patogeneza i możliwości diagnostyczno-terapeutyczne

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Abstract

Non-alcoholic fatty liver disease is the most common chronic disease of the liver in children. This term encompasses a wide spectrum of disorders of liver tissue: from mild steatosis to non-alcoholic steatohepatitis; the abnormalities may eventually lead to liver cirrhosis and the development of hepatocellular carcinoma. The prevalence of non-alcoholic fatty liver disease is estimated to be approximately 25% in the general population, while the rate for the paediatric population ranges from 7.6% to as many as 34.2% in obese children. In accordance with the current definition of non-alcoholic fatty liver disease, the condition is diagnosed after steatosis has been confirmed using histopathological or imaging methods and secondary causes of liver damage have been excluded, such as alcohol abuse, the use of hepatotoxic medicines and congenital or infectious diseases. The disease is most commonly diagnosed in the population of overweight and obese children; it often coexists with insulin resistance. There are reports of the impact of genetic factors on the development of the condition. Clinical signs and symptoms are non-specific: hepatomegaly, malaise and a sense of discomfort in the abdominal cavity. Basic diagnostic investigation includes aminotransferase test and abdominal ultrasound examination. Researchers pay much attention to non-invasive diagnostic methods such as serological markers and fibrosis scores. Due to the fact that no pharmacotherapy has a confirmed efficacy in the treatment of non-alcoholic fatty liver disease, the most important prevention and treatment methods in the paediatric population are lifestyle modification, physical activity and a balanced diet. The utility of vitamin E, ursodeoxycholic acid and metformin has not been confirmed by research. Due to the high prevalence of the condition and the risk of complications, familiarity with the problem is important particularly among general practitioners.

Keywords: fatty liver, children, non-invasive diagnostic methods

Streszczenie

Niealkoholowa stłuszczeniowa choroba wątroby to najczęściej występująca przewlekła choroba wątroby u dzieci. Pod tym pojęciem kryje się szerokie spektrum zaburzeń dotyczących tkanki wątrobowej – od łagodnego stłuszczenia do niealkoholowego stłuszczeniowego zapalenia wątroby; zmiany te ostatecznie mogą prowadzić do marskości wątroby i rozwoju raka wątrobowokomórkowego. Częstość występowania opisywanej jednostki w populacji ogólnej szacuje się na około 25%, wśród populacji dziecięcej od 7,6% do aż 34,2% w grupie dzieci otyłych. Zgodnie z aktualną definicją niealkoholową stłuszczeniową chorobę wątroby rozpoznajemy, potwierdzając stłuszczenie metodami histopatologicznymi lub obrazowymi i wykluczając wtórne przyczyny uszkodzenia wątroby, takie jak nadużywanie alkoholu, stosowanie leków hepatotoksycznych, schorzenia wrodzone czy zakaźne. Najczęściej chorobę rozpoznaje się w populacji dzieci z nadwagą i otyłych, często współistnieje z nią insulinooporność. Istnieją doniesienia o wpływie czynników genetycznych na jej rozwój. Objawy kliniczne są niespecyficzne, obejmują hepatomegalię, złe samopoczucie, uczucie dyskomfortu w jamie brzusznej. W podstawowej diagnostyce uwzględnia się aktywność aminotransferaz oraz badanie ultrasonograficzne jamy brzusznej. Dużą uwagę badaczy przykuwa diagnostyka nieinwazyjna – markery serologiczne, skale do oceny włóknienia. Wobec braku potwierdzenia skuteczności farmakoterapii modyfikacja stylu życia, aktywność fizyczna i zbilansowana dieta to najważniejsze sposoby profilaktyki i leczenia niealkoholowego stłuszczenia w populacji dziecięcej. Stosowanie witaminy E, kwasu ursodezoksycholowego czy metforminy nie znalazło potwierdzenia w badaniach naukowych. Ze względu na częstość występowania i ryzyko powikłań znajomość zagadnienia jest ważna zwłaszcza wśród lekarzy pierwszego kontaktu.

Słowa kluczowe: stłuszczenie wątroby, dzieci, diagnostyka nieinwazyjna

DEFINITION AND PATHOGENESIS

The term "non-alcoholic fatty liver disease" (NAFLD) encompasses a wide range of pathological processes in the liver, from plain steatosis to non-alcoholic steatohepatitis (NASH), which occur in individuals who do not abuse alcohol and in whom other possible causes of damage to the organ have been excluded, such as viral infections, autoimmune hepatitis, metabolic diseases, use of hepatotoxic medicines or malnutrition. Progression of pathological processes can lead to the end of the continuum of liver tissue abnormalities: advanced fibrosis, cirrhosis and even hepatocellular carcinoma[1,2]. In accordance with the histopathological definition of the disease, steatosis may be diagnosed if lipid accumulation is present in at least 5% of hepatocytes[3]; however; the current recommendations on NAFLD diagnosis stipulate that it is sufficient to confirm the presence of steatosis on imaging studies[4]. Non-alcoholic steatohepatitis is diagnosed when steatosis leads to hepatic cell damage manifesting with apoptosis, ballooning degeneration, periportal inflammation and subsequent development of fibrosis.

Precise mechanisms responsible for disease progression in patients with NAFLD are not known; however, lipotoxicity and the activity of factors originating from the adipose tissue and the intestines are considered to be of key importance. Increased deposition of fat is caused by an increased intake of free fatty acids (FFA), their decreased oxidation in the liver and by an increased *de novo* lipogenesis. As the ability of hepatocytes to metabolise FFA is exceeded, free oxygen radicals are formed, oxidative stress is increased and toll-like receptor (TLR) signalling pathways are activated. As a result, inflammation develops and cells are damaged and die. Oxidative stress is responsible for the stimulation of proinflammatory cytokine production and the activation of hepatic stellate cells (HSC), which leads to an increased production of extracellular matrix and liver fibrosis. The development of inflammation and fibrosis is also contributed to by intestinal endotoxins by activating the TLR of hepatocytes and Kupffer cells (KC)[5,6].

Due to its function, the liver is considered to be an important organ responsible for body immunity, which acts as a filter against antigens coming from the blood and the gastrointestinal tract and against toxins and takes part in their removal from the system. NAFLD, as other liver diseases, is probably the result of interaction between cells of the immune system. Hepatic cells of the immune system include the Kupffer cells mentioned above and lymphocytes, including natural killer cells (NK) and natural killer T-cells (NKT). Free fatty acids supplied with food bind to TLR located at the surface of cells, activating immune response. Cytokines released from adipose tissue may act in a similar way: tumour necrosis factor α (TNF-α) activates KC through their surface receptors. Kupffer cells may activate NK through NKG2D receptors and interleukins (IL-12, IL-18); however, the precise mechanism of KC activation in NAFLD pathogenesis remains unclear[5].

Many studies indicate that NKT modulate the immune response and fibrinogenesis-stimulating mechanisms associated with liver diseases; there is an increasing body of evidence for their role in NAFLD pathogenesis. In addition, decreased levels of NKT in both liver biopsy specimens and peripheral blood have been demonstrated in affected patients. Based on the available reports it may be assumed that there is a negative correlation between the liver population of NKT and the severity of histopathological abnormalities in the liver[7]. The mechanism whereby liver steatosis leads to decreased numbers of NKT remains unclear. The possible mechanism is stimulation of apoptosis. The activated NKT may stimulate hepatocyte apoptosis through an increased expression of FAS ligand, which most probably plays a role in NASH pathogenesis.

Initially, the process of liver steatosis and its progression to NASH was explained using a two-hit theory: insulin resistance present particularly in obese individuals and the associated insulin level increase lead to an increased deposition of lipids in liver tissue (the first hit). The second hit was considered to involve changes caused by oxidative stress, which damages hepatocytes overloaded with lipids. Currently, research on the genetic aspects of the development of NAFLD warrants the assumption of a multiple-hit hypothesis. The most important genes associated with the development of NAFLD include *PNPLA3* (patatin-like phospholipase-containing domain 3). Polymorphism of a single nucleotide present in the gene probably leads to the development of small-droplet steatosis by changing the properties of an enzyme formed. Polymorphism of the *TM6SF2* gene (transmembrane 6 superfamily member 2) also leads to increased accumulation of fat in the liver by reducing the secretion of very low density lipoproteins (VLDL). The *GPR120* (G-protein-coupled-receptor 120) allele, which codes an appropriate receptor present at the surface of KC, among other cells, is responsible for the second hit according to the hypothesis presented above by weakening the anti-inflammatory function of the receptor on the surface of KC[8].

EPIDEMIOLOGY AND RISK FACTORS

In the context of the growing global problem of overweight and obesity, NAFLD is the most common liver pathology both among adults and children[8]. Due to the lack of simple, inexpensive and non-invasive examination methods providing a confident diagnosis, it is difficult to make a definitive estimate of NAFLD prevalence. According to a review and meta-analysis of the available sources, the global prevalence of NAFLD estimated based on imaging studies is 25.24%[4]; according to British authors, the prevalence of NAFLD in the population aged from 1 to 19 is 7.6%; among obese patients, the prevalence is 34.2%[9].

According to Polish autopsy data from 2000–2009, among children who died due to trauma, liver steatosis was found in 4.2% of cases and over half of the examined children were obese (54.5%)[10]. The most important risk factors

 DOI: 10.15557/PiMR.2019.0042

Cause of liver damage	Suggested additional laboratory tests
Infectious factors	Anti-HCV antibodies HBsAg, anti-HBc Anti-EBV VCA IgM, IgG, anti-EBNA Anti-CMV IgM, IgG Anti-HSV-1, -2 IgM, IgG Antibodies against enteroviruses
Haemochromatosis	Serum iron and ferritin level, TIBC
Wilson's disease	Serum copper and ceruloplasmin level; copper excretion in a 24-hour urine collection test
Coeliac disease	Anti-endomysial autoantibodies and IgA anti-tissue transglutaminase autoantibodies and total IgA level
α1-antitrypsin deficiency	Serum α1-antitrypsin level
Cystic fibrosis	Sweat test
Other metabolic diseases	Plasma amino acid profile, urine organic acid profile, blood acylcarnitine profile, free fatty acid level
Autoimmune hepatitis	Serum γ-globulin level; titre of ANA, SMA, anti-LKM1 and other antibodies (e.g. LC-1 or anti-SLA/LP)
HCV – hepatitis C virus; **HBsAg** – hepatitis B surface antigen; **anti-EBV VCA** – antibodies against the viral capsid antigen of Epstein–Barr virus; **anti-EBNA** – antibodies against Epstein–Barr nuclear antigen; **CMV** – cytomegalovirus; **HSV** – herpes simplex virus; **TIBC** – total iron binding capacity; **ANA** – antinuclear antibodies; **SMA** – smooth muscle antibodies; **anti-LKM1** – anti-liver-kidney microsome antibodies type 1; **LC-1** – liver cytosol antigen 1; **anti-SLA/LP** – anti-soluble liver antigen/liver pancreas antigen.	

Tab. 1. Proposed additional tests in differential diagnosis of NAFLD

for NAFLD, based on the results reported above, are overweight and obesity; based on body mass index (BMI), individuals above the 85th and 95th centile on growth charts for age and sex are classified as overweight and obese, respectively. In addition, it is considered that children coming from families with the problem of obesity, insulin resistance or type 2 diabetes are characterised by a higher risk of NAFLD[11]. In the paediatric population, a higher prevalence of the disease was found in children aged over 10 than in younger children; this is probably due to smaller parental control over diet and hormonal changes characteristic for puberty such as increased blood triglyceride levels and the associated increase in insulin resistance. Approximately 25% of patients with NAFLD display the signs of NASH; in this group, fibrosis or cirrhosis can be expected to occur in approximately 10–20% of individuals[12].

CLINICAL SIGNS AND SYMPTOMS, LABORATORY TESTS

The majority of children with NAFLD/NASH do not have any specific signs or symptoms. Hepatomegaly, malaise and abdominal discomfort are common observations[10,13]. Acanthosis nigricans can be observed on the skin of patients with elevated insulin levels. Based on the observations concerning the causes of and risk factors for NAFLD, one always should make anthropometric measurements; as the waist circumference increases, so does the risk of signs of liver steatosis on ultrasound[14].

There are no laboratory tests for NAFLD. Increased aminotransferase and gamma-glutamyl transpeptidase levels and hypertriglyceridaemia are often observed. Despite little sensitivity, an alanine aminotransferase (ALT) test showing high readings should draw the attention of the examiner, who should perform further tests[14]. The upper limit of normal for ALT remains a controversial issue; the cut-off values assumed by laboratories are probably too high[15]. According to the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) consensus regarding NAFLD diagnosis in children and adolescents, the following should be included in laboratory testing[13]:

- basic tests: complete blood count, alanine and aspartate aminotransferases, gamma-glutamyl transpeptidase, alkaline phosphatase, bilirubin, glucose, insulin, urea, electrolytes, blood coagulation test;
- lipid profile: total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL);
- oral glucose tolerance test (OGTT), glycated haemoglobin, HOMA-IR;
- thyroid hormones.

DIFFERENTIAL DIAGNOSIS

In accordance with the definition of non-alcoholic fatty liver disease, the diagnosis can be made after other causes of liver damage have been excluded such as Wilson's disease, coeliac disease, autoimmune hepatitis, α1-antitrypsin deficiency or haemochromatosis. Alcohol abuse, the use of hepatotoxic medicines, parenteral nutrition and malnutrition should also be excluded. The suggested laboratory tests depending on the suspected aetiology are presented in Tab. 1.

HISTOPATHOLOGICAL AND IMAGING STUDIES

Despite evident progress in the development of non-invasive diagnostic imaging methods and studies on serum markers, the reference method for the diagnosis of NAFLD remains liver biopsy. Histopathological examination allows one to assess the cytoarchitecture of liver parenchyma and differentiate between plain steatosis and NASH. Typically, a biopsy specimen of a patient with NASH shows large

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Investigated process	Marker	Existing observations
Inflammation[19,20]	Adipokines (adiponectin, resistin, visfatin)	A negative correlation was found between the level of adiponectin and HOMA-IR; the level of adiponectin was also lower in patients with histologically confirmed NASH. There was no relationship between the severity of inflammation and resistin level; a relationship between resistin level and TNF-α level was only identified, particularly in patients without obesity. In another study, the resistin level was lower in a group with NASH compared to a group with plain steatosis. Visfatin level was higher in obese patients, but lower in patients with NASH compared to the group with plain steatosis (link with IL-6)
	TNF-α	The level of TNF-α was higher in patients with NAFLD compared to non-obese and obese patients without NAFLD
	IL-6, IL-8	The role of IL-6 has not been fully accounted for; higher levels have been found in obese patients and patients with plain steatosis and lower levels were observed in patients with NASH compared to those with plain steatosis. The IL-8 level was higher in patients with NASH
Cell death (apoptosis)[21]	Cytokeratin 18	Elevated cytokeratin 18 level is predictive for NASH (70% sensitivity and 83.7% specificity)
Oxidative stress[22]	TBARS	Elevated TBARS level in patients with NAFLD both with diagnosed type 2 diabetes and without it
Fibrosis[23]	ELF panel: hyaluronic acid, procollagen type III N-terminal propeptide, tissue inhibitor of metalloproteinase I	High sensitivity and specificity of the test in estimating the degree of fibrosis in patients with NAFLD

HOMA-IR – homeostatic model assessment for insulin resistance; **TNF-α** – tumour necrosis factor; **IL** – interleukin; **TBARS** – thiobarbituric acid reactive substances; **ELF** – European Liver Fibrosis.

Tab. 2. Utility of serum markers in the assessment of processes in NAFLD pathophysiology

droplet steatosis with nucleus displacement towards the periphery of the cell, ballooning degeneration (diagnosed less frequently in children than in adults), inflammatory infiltration and, less commonly, periportal fibrosis, Mallory bodies and megamitochondria[16].

The moment of biopsy collection is a controversial issue. From a practical point of view, it seems rational to start with a dietary intervention and prescribing physical activity; if there is no improvement regarding laboratory and imaging findings, a biopsy specimen should be collected within a few months' time. The limitations of biopsy should also be taken into account such as invasiveness, possible complications (usually pain, haemorrhage, perforation of other organs) and the level of experience of the pathologist assessing the specimen[17].

The most commonly performed imaging procedure, treated as a screening method, is ultrasound scan. Typically, bright, hyperechoic areas (compared to the image of the neighbouring right kidney and spleen) consistent with steatosis are observed. The estimated sensitivity of the method in the diagnosis of NAFLD in adults is 60–96% and the estimated specificity is 84–100%[18]. Both parameters rise if steatosis affects >20% of the organ. Unlike ultrasound, apart from qualitative assessment, computed tomography and magnetic resonance imaging make it possible to perform quantitative analysis of fatty liver tissue. In non-invasive diagnosis of liver problems, particularly in patients with a normal body mass, the FibroScan device is also used, which measures the degree of fibrosis based on elastography. However, with relevance to children, there is no conclusive research yet confirming the efficacy of this method in all age groups.

ASSESSMENT OF SERUM BIOMARKERS IN NAFLD

Due to the invasive nature of liver biopsy, which is a diagnostic standard for NAFLD, much attention is being paid to studies on serum markers of hepatocyte damage which could be used to perform population screening and assess the course of the disease in patients with diagnosed steatosis. It seems to be of particular importance to be able to differentiate, based on biomarker analysis, between plain steatosis and NASH and to assess the degree of fibrosis. In Tab. 2 markers are grouped depending on the processes which they correspond to in the pathogenesis of NAFLD.

For fibrosis assessment, apart from the specific markers mentioned above, the use of tests composed of a number of laboratory indicators is proposed. As studies show, the indices used for adult patients or those with hepatitis C such as NFS (NAFLD fibrosis score) or FIB-4 (Fibrosis-4 index) are not reliable for children with NAFLD. In a study comparing the available indices (2014) it was only APRI (AST to platelet ratio index) that had a good predictive value for the detection of fibrosis ($p = 0.001$). At the next stage of the study, the efficacy of the reported indices was compared in the differentiation of the degree of fibrosis; no statistically significant differences in APRI values were found between groups with mild and advanced fibrosis[24].

The highest hopes are placed in the American-Italian PNFS score (paediatric NAFLD fibrosis score). The calculations take into account the levels of alanine aminotransferase, alkaline phosphatase and gamma-glutamyl transpeptidase and platelet count. The area under the ROC curve (area under receiver operating characteristic, AUROC) for

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PNFS was 0.74 (95% confidence interval, CI: 0.66–0.82) and was higher than AUROC for APRI, NFS and FIB-4, which makes PNFS the most useful tool among the currently available ones[25].

PREVENTION AND TREATMENT

Lifestyle modification remains the priority in the treatment of non-alcoholic fatty liver disease. The available studies indicate a beneficial effect of physical activity and diet on reducing ALT levels and improving the histopathological findings in children with NAFLD. The recommendations of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN) emphasise the harmful effects of sugar-sweetened beverages on body mass. Patients should have a Mediterranean diet with restricted amounts of red meat and simple sugars and with large amounts of fish, seafood, fruit and vegetables. The time spent in front of a computer or TV screen should be reduced (to less than 2 hours daily) in favour of moderate and intensive physical activity[26].

There are many studies on the efficacy of medicines and dietary supplements in the treatment of NAFLD. Vitamin E and metformin have raised particular interest; a statistically significant improvement of the histological image and a lower risk of the development of NASH in patients receiving vitamin E compared to a placebo-taking group have been observed[27]. Due to inconclusive data regarding the risk of taking high doses of vitamin E in adults (increased mortality, elevated risk of cardiovascular events), vitamin E is ultimately not recommended. In the same study, metformin has not been found to be effective in the reduction of steatosis and ALT levels. Similar conclusions were made by researchers who studied the benefits of using ursodeoxycholic acid, docosahexaenoic acid and probiotics[26].

In extraordinary cases: in teenagers with a BMI of >35 kg/m^2 and comorbidities such as type 2 diabetes or obstructive sleep apnoea, bariatric surgery may prove beneficial[28].

In all children with diagnosed NAFLD, lipid levels should be regularly assessed and arterial pressure should be monitored. It is suggested that upon diagnosis of fatty liver disease, serum glucose or glycated haemoglobin (HbA_{1c}) level should be assessed; the test should be repeated at least once a year due to the elevated risk of prediabetes and diabetes[29]. The importance of preventative action and conversations regarding the possible influence of medicines on liver function, the harmful effects of tobacco smoking, the need to avoid infection with hepatotropic viruses and to have one's serological status assessed is also stressed, particularly in the general practitioner's office.

CONCLUSION

The knowledge of issues regarding non-alcoholic fatty liver disease in children, in the context of its high prevalence, is very important in everyday practice of a family doctor and paediatrician. All preventative actions and the majority of preliminary diagnosis methods for NAFLD are available in primary care settings.

The diagnosis of NAFLD is based on the confirmation of steatosis (using histopathological or imaging methods) and exclusion of secondary causes of excessive fat accumulation in hepatocytes. Clinical signs and symptoms are non-specific, therefore, studies on non-invasive methods for the diagnosis and assessment of liver steatosis and fibrosis as prognostic factors seem to be promising. Despite huge progress in medicine, currently, there is no specific treatment for NAFLD apart from non-pharmacological methods: physical activity and appropriate nutrition.

Conflict of interest

The authors do not report any financial or personal affiliations to persons or organisations that could adversely affect the content of or claim to have rights to this publication.

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