Liver steatosis in Polish children assessed by medico-legal autopsies

Marta Rorat, Tomasz Jurek, Ernest Kuchar, Leszek Szenborn, Wojciech Golema, Agnieszka Halon

Wroclaw, Poland

Original article

Background: **Cases of non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) are increasing in the pediatric population. Their growing prevalence coincides with the obesity epidemic. Assessment of the incidence requires liver biopsies on a representative population sample, which are hampered by the absence of indications for invasive examination on children without clinical symptoms. The aim of the current study was to assess the incidence of liver steatosis in the population of children up to 18 years old from Lower Silesia.**

Methods: **We retrospectively reviewed 342 medico-legal autopsy reports from 2000 to 2009. We separated a group of 256 children whose death was caused by trauma. Liver steatosis was diagnosed according to the results of histopathological examinations and typical macroscopic imaging.**

Results: **In the 265 children who died from trauma, liver steatosis was reported in 11 (4.2%) children (6 boys) aged between 6 months and 18 years old. Six of the 11 children (54.5%) were found to be overweight. In all 342 children, steatosis was found in 18 (5.3%) children (13 boys), while NASH was diagnosed in 1 (0.3%). Excess body weight was observed in 55.6% (10/18) of children with steatosis.**

Conclusions: **Liver steatosis can occur at any age, even in infancy. Being overweight is a very important risk factor. Gross examination of the liver is insufficient for the diagnosis of steatosis because of its lower sensitivity and specificity. Verification of liver steatosis requires reference histopathological examination.**

World J Pediatr 2013;9(1):68-72

Key words: **autopsy;**
liver steotosis;
non-alcoholic fatty liver disease;
obesity

Introduction

Non-alcoholic fatty liver disease (NAFLD) is currently the most common liver pathology in children and adults.[1,2] It is estimated to affect about 20%-30% of adults in developed countries. NAFLD belongs to the group of so-called emerging diseases, which coincides with the obesity epidemic. Overweight and obesity with insulin resistance are the biggest risk factors for the development of this disease.[1,3,4]

NAFLD usually proceeds as a mild, simple liver steatosis, but it may develop into non-alcoholic steatohepatitis (NASH) in some patients (about 20%-30%) or into cirrhosis and hepatocellular carcinoma in fewer cases.[1,3,5,6]

The incidence of liver steatosis, especially among children, is still difficult to assess because of the small number of population studies. Assessment of the incidence of steatosis is usually based on the results of serum laboratory tests (transaminase activity, lipid metabolism) and ultrasonography, and only occasionally on the results of liver biopsies.[3,7-10] The standards adopted differ from one another, such as the methods of determining concomitant obesity and selecting age groups, which affects the differences in the assessment of disease incidence.[3,5,7,10] In general, diagnostic tests are implemented in the presence of clinical or laboratory symptoms of a liver disease, which means that only symptomatic cases are diagnosed. The actual scale of the pathology can only be assessed on the basis of studies of a population group.

In Poland, liver steatosis in children is underestimated, and excess weight as well as obesity are still treated as minor threats or neglected. Furthermore, there is no epidemiological data to represent the scale of the

Author Affiliations: Department of Forensic Medicine (Rorat M, Jurek T, Golema W), Department of Pediatrics and Infectious Diseases (Kuchar E, Szenborn L), Department of Pathological Anatomy (Halon A), Wroclaw Medical University, Poland

Corresponding Author: Marta Rorat, Department of Forensic Medicine, Medical University of Wroclaw, Mikulicza Radeckiego 4, Wroclaw 50-368, Poland (Tel: +48-71-784-14-74; Fax: + 48-71-784-00-95; Email: marta.rorat@gmail.com)

doi: 10.1007/s12519-012-0387-8

problem in this country.

The aim of the current study was to analyze the incidence of liver steatosis in the population of children and adolescents up to 18 years old from Lower Silesia in Poland, without limiting the study group to cases with pre-existing disease symptoms.

Methods

A retrospective review was made of 342 medico-legal reports on autopsies performed at the Department of Forensic Medicine in Wroclaw from 2000 to 2009, concerning patients from the age of 1 month to 18 years old. Liver steatosis was diagnosed according to the results of histopathological examinations (hematoxylin and eosin staining) and/or the organ's appearance (enlargement of the liver, yellow colour, blurring of the structure) during gross examination. Histological assessment of the liver was performed in 128/342 (37.4%) children. Specimens were taken from the right lobe of the liver and interpreted by a pathologist. Steatosis was analysed according to Dixon's scale for the liver by another pathologist specializing in liver pathology. The adopted histopathological criterion, ≥5% of fatty hepatocytes, was identical to those used in other studies.[1,3,4]

A group of people who died from violent causes were separated in order to assess the incidence of the clinically-silent disease. The group also included deaths for which the direct cause has not been established. Those who could have affected the histological picture of the liver (alcohol, medicines and drugs, sepsis) were excluded. Supplementary analysis of steatosis occurrence has also been conducted for the group of children over two years old.

The group of children who died from violent causes was compared to all children, taking the age of the subjects into account. In accordance with the World Health Organization (WHO) definition, overweight was defined as a BMI between the 85th and 95th percentile (for gender and age, respectively) while obesity was defined as BMI ≥95th percentile.[11,12] The results were prepared using version 3.5.1 of the Epi Info database.

Results

Among the 342 children, subjected to a medico-legal autopsy in the period of 2000-2009, 108 were girls and 234 boys. Liver steatosis was found in 18 children (13 boys and 5 girls), aged from 6 months to 18 years (Fig. 1). Diagnosis was based on histological examination in 10 cases: 9 involved diffuse, macrovesicular, fatty degeneration of the liver (3 degeneration of the 1st degree, 2 of the 3rd degree and 4 the 4th degree on the Dixon scale. One case found with microvesicular fatty degeneration of the liver (3rd degree on the Dixon scale) was diagnosed. In one case, we found signs of NASH (Fig. 1). Of the 326 weighted chidlren, 42 were overweight, accounting for 12.9% (boys: 29/222, 13.1% and girls: 13/104, 12.5%). Among them, 4.6% (boys: 8/222, 3.6%, girls: 7/104, 6.7%) were obese. Excessive body weight together with liver steatosis was found in 10/18 (55.6%) children, including one with obesity; in the 57 (17.5%) children with excess body weight, 10 had liver steatosis.

For the 265 children with the death cause of a violent nature (Fig. 2), the most common causes of death were road accident 99/265 (37.4%), hanging 27/265 (10.2%), drowning 25/265 (9.4%), and falling from a height 23/265 (8.7%). In this group, liver steatosis was found in 11/265 (4.2%) children (6 boys, 5 girls) aged from 15 months to 18 years. Histological examination revealed diffuse, macrovesicular fatty degeneration of the liver in three children. Two of them were steatosis 3rd degree and one 4th degree according to the Dixon scale. Typical morphology during gross examination showed advanced liver steatosis in the other eight cases (Fig. 1). In this group, 13.8% (24 boys and 11 girls) were overweight, and 3.95% (6 boys, 4 girls) obese. In the 11 children diagnosed with liver steatosis, 54.5% (6/11) were overweight; and in the 45 children with excessive body weight, 6 (13.3%) had liver steatosis.

In both groups, there were false positive diagnoses based on the gross examination (macroscopically recognized liver steatosis was not confirmed by histopathological examination in 2 children in the violent death group and 5 in the group of all children), which were excluded from further analysis. Microscopic examination of the liver was conducted on 128/342 (37.4%) children, of whom only 62/265 (23.4%) died from violent causes. The prevalence of steatosis was 4.8% (3/62) in the violent death group,

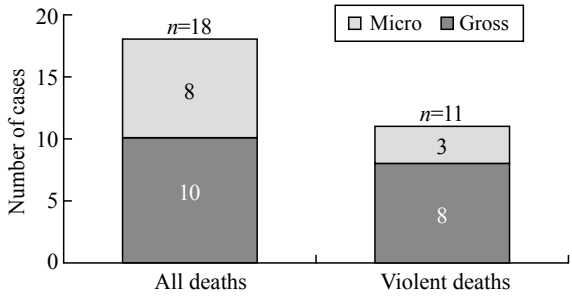

Fig. 1. Number of cases with microscopic and gross examination in violent and all death cases.

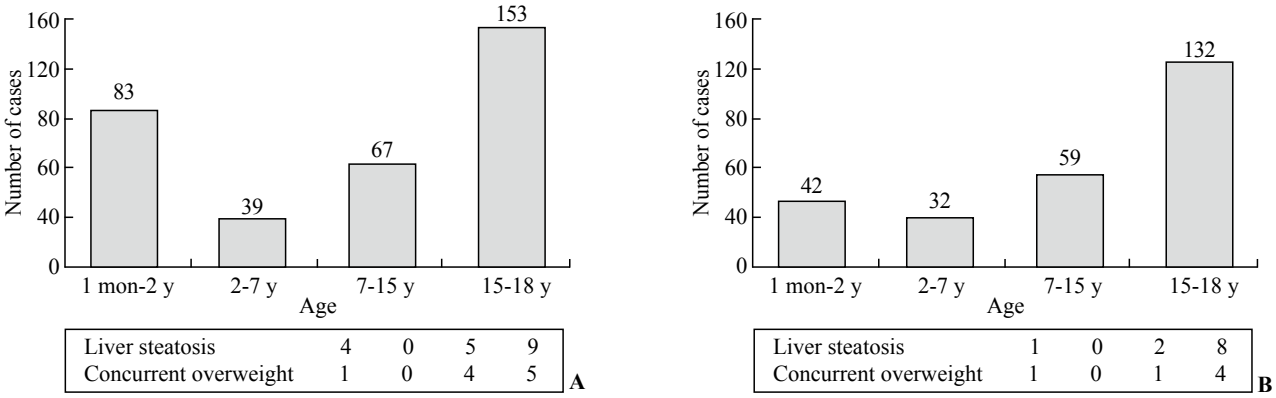

Fig. 2. The age distribution of the group, the incidence of liver steatosis and concurrent overweight in all deaths (**A**) and violent death group (**B**).

and 7.8% (10/128) in all children.

In children aged 2 years and above, the prevalence of steatosis was 4.67% (10/214) in the violent death group, and 5.36% (14/261) in all children.

Discussion

Liver steatosis seems to be more prevalent in children than in other populations shown by population-based non-invasive epidemiological studies because most analyses are based on serum transaminase activity and liver ultrasonography. Hence, only a few studies are based on histopathological examination of the liver.[3,7-9]

In Poland, we conducted the first study assessing the incidence of liver steatosis in children and adolescents up to 18 years old who were subjected to medico-legal autopsy. The incidence of liver steatosis in the population of healthy children in this country was 4.2%, which is similar to the global rate. The incidence of liver steatosis in Asia and the USA varies between 2.6%-9.6%.

Based on autopsy studies, Schwimmer et al[3] reported that the incidence of liver steatosis in children aged 2-19 years in San Diego was 9.6%. The highest incidence of fatty liver was 38% in obese children. Tominaga et al[7] reported that the incidence of fatty liver in Japanese children aged 4-12 years was 2.6% estimated by characteristic ultrasound imaging. In another study with the same method, they found the incidence of NAFLD was 4.4% in children aged 6-15 years.[8]

The high incidence of liver steatosis in children is associated with the epidemic of obesity. Obesity is currently one of the major health risks affecting children and adolescents.[11,13] According to the WHO report in 2010, about 43 million children under the age of 5 years were overweight, and obesity affects nearly 15% of children aged 6-19 years.[13] In the violent death group of this study, we found 54.5% of children with liver steatosis were overweight. In the group of all deaths with confirmed steatosis, excess body weight was found in 55.6% of the children. Overweight and obesity were less often associated with the development of steatosis. Fatty liver was found in 13.8% of overweight children in the violent death group, and 17.5% in all children. The incidence of liver steatosis in obese children in the USA and Europe was within the range of 16%-77% according to the study method adopted.[3,9,10,14-16]

The lower rate of liver steatosis in the Polish population compared to the United States may be due to a lower incidence of excess weight and obesity. Studies have shown that 11.1% of boys and 14.4% of girls aged 7-18 years in Poland are overweight whereas 4.8% of boys and 2.5% of girls are obese.[17] In children and adolescents aged 2-19 years in the USA, 16.9% are obese and 31.7% overweight. In addition, 9.5% of infants and toddlers are obese.[18] Thus, in analysis of the differences in the incidence of liver steatosis, we should consider factors such as lifestyle, diet and ethnic background as well as diseases accompanied by insulin resistance like polycystic ovary syndrome.[1,3] In recent years, genetic factors determining NAFLD, e.g. the *PNPLA3* gene, have been thoroughly investigated.[19,20] Childhood obesity is associated with a higher risk of obesity in adulthood, disability and premature death.[21] Studies have shown that the occurrence of serious liver damage may be due to obesity. Natural history and prognosis of fatty liver in children remain unknown. In children with NASH, however, liver fibrosis is often observed.[22,23] NAFLD is now recognized as the most common cause of cryptogenic cirrhosis.[5,24,25]

Schwimmer et al[4] investigated 100 patients aged 2-18 years with clinically diagnosed and histopathologically confirmed steatosis (92% were obese, 6% overweight). Histological examination of the liver showed that simple steatosis occurred in only

16% of the children, but as many as 8% were diagnosed with advanced fibrosis and 3% with cirrhosis. The study pointed out the lack of diagnostic criteria for NAFLD/NASH in children. The previously described inflammatory and fibrotic changes differ from those observed in adults. Feldstein et al[22] performed liver biopsies in 29 patients with NAFLD, of whom five were subjected to follow-up monitoring and examination. Fibrotic progression was reported in four children. During the follow-up period, two children developed cirrhosis requiring a transplant and two died of decompensated cirrhosis.

The degrees of steatosis, fibrosis, and hepatitis were found not to be correlated with clinical symptoms.[26] In most children, NAFLD is asymptomatic and develops undetectably. The diagnosis of the disease should be based on routine laboratory tests (elevated serum transaminases, alanine aminotransferase>aspartate aminotransferase, increase in gamma-glutamyl transferase and alkaline phosphatase activity, dyslipidemia) or ultrasound/MRI examinations made for any other reasons. In rare cases, it can be the cause of sudden death in young adults.[1]

Only histological examination can confirm the diagnosis and assess the severity of steatosis.[1,2,24,25] Liver biopsies are an expensive, invasive method, with the substantial risk of complications. Thus, in patients with NAFLD, it is only performed if there is strong evidence suggesting liver damage.[2] Therefore, it cannot be used for screening.[2,27]

Unfortunately, to date, there are no reliable replacement markers or imaging examinations that histological assessment can provide such as bioptate, differences in the degree of liver steatosis, distinction between simple steatosis and steatohepatitis, assessment of fibrosis, inflammation and cirrhosis, and even detection of other coexisting liver pathologies.[27]

Non-invasive surrogate markers are characterized by insufficient sensitivity and specificity. For example, ultrasonography, although cheap and quick, is not very useful in cases of mild steatosis since the result depends on the examiner's skills and experience, the quality of the equipment, and other factors (e.g. characteristics of the body – a large amount of fatty tissue on the abdomen may produce false positive results). Hence, it is not suitable for diagnosing the severity of the disease.[1-3] The sensitivity and specificity of transaminase activity in the diagnosis of NAFLD in children are not determined.[2] MRI is characterized by a higher sensitivity.[28] Because of many controversies linked with non-invasive tests assessing the incidence of liver steatosis in children and significant limitations on their ability to perform liver biopsies, the optimal approach would be to conduct population studies based on autopsy studies.

To assess the incidence and severity of liver steatosis in the population group regardless of the cause of death and relationship of the disease to obesity and excess weight, we observed the occurrence of fatty liver in children of 2 years old. In very few studies on this age group, attention was concentrated on the presence of genetic and metabolic diseases as the background for the occurrence of fatty liver.[29]

The limitation of our study is a relatively small size. In addition, histological assessment of the liver was performed in 128 children, preventing in some instances verification of gross examination of the liver. Because of the specificity of forensic autopsy, histological examination of the liver is not routinely carried out when the cause of death is obvious. Additional tests depend on the procurator's order. Histopathological examination is carried out if the cause of death remains unclear or if pathological changes are atypical during the gross examination. In the two false positive cases from the violent death group in our study, macroscopic imaging was difficult to interpret because of coexisting pathologies (one was diagnosed with tyrosinemia and another with good pasture syndrome). Moreover, we underestimated the incidence of steatosis by overlooking cases of mild steatosis in the group where only gross examination was performed. A prospective study of histological results for the liver in each child died non-pathologically would be of greater value. Another limitation of the study is the inability to perform a differential diagnosis of liver steatosis because of lack of data from anamnesis, growth history and limited possibilities of post-mortem diagnosis of infectious, autoimmune, and metabolic diseases.

In conclusion, liver steatosis is relatively frequent in Polish children and adolescents in Lower Silesia. Overweight is a risk factor. Gross examination of the liver is insufficient for the diagnosis of steatosis because of its low sensitivity and specificity. Verification of liver steatosis requires reference histopathological examination.

Funding: None.
Ethical approval: Not needed.
Competing interest: None declared.
Contributors: Rorat M and Kuchar E proposed the study and with Jurek T wrote the first draft. Rorat M and Golema W aquired the data. All authors contributed to the design and interpretation of the study. Rorat M is the guarantor.

References

1 Yu EL, Schwimmer JB, Lavine JE. Non-alcoholic fatty liver disease: epidemiology, pathophysiology, diagnosis and treatment.

Paediatr Child Health 2010;20:26-29.
2 Patton HM, Sirlin C, Behling C, Middleton M, Schwimmer JB, Lavine JE. Pediatric nonalcoholic fatty liver disease: a critical appraisal of current data and implications for future research. J Pediatr Gastroenterol Nutr 2006;43:413-427.
3 Schwimmer JB, Deutsch R, Kahen T, Lavine JE, Stanley C, Behling C. Prevalence of fatty liver in children and adolescents. Pediatrics 2006;118:1388-1393.
4 Schwimmer JB, Behling C, Newbury R, Deutsch R, Nievergelt C, Schork NJ, et al. Histopathology of pediatric nonalcoholic fatty liver disease. Hepatology 2005;42:641-649.
5 Preiss D, Sattar N. Non-alcoholic fatty liver disease: an overview of prevalence, diagnosis, pathogenesis and treatment considerations. Clin Sci (Lond) 2008;115:141-150.
6 Patton HM, Lavine JE, Van Natta ML, Schwimmer JB, Kleiner D, Molleston J. Nonalcoholic Steatohepatitis Clinical Research Network. Clinical correlates of histopathology in pediatric nonalcoholic steatohepatitis. Gastroenterology 2008;135:1961-1971.e2.
7 Tominaga K, Kurata JH, Chen YK, Fujimoto E, Miyagawa S, Abe I, et al. Prevalence of fatty liver in Japanese children and relationship to obesity. An epidemiological ultrasonographic survey. Dig Dis Sci 1995;40:2002-2009.
8 Tominaga K, Fujimoto E, Suzuki K, Hayashi M, Ichikawa M, Inaba Y. Prevalence of non-alcoholic fatty liver disease in children and relationship to metabolic syndrome, insulin resistance, and waist circumference. Environ Health Prev Med 2009;14:142-149.
9 Chan DF, Li AM, Chu WC, Chan MH, Wong EM, Liu EK, et al. Hepatic steatosis in obese Chinese children. Int J Obes Relat Metab Disord 2004;28:1257-1263.
10 Radetti G, Kleon W, Stuefer J, Pittschieler K. Non-alcoholic fatty liver disease in obese children evaluated by magnetic resonance imaging. Acta Paediatr 2006;95:833-837.
11 Nader PR, O'Brien M, Houts R, Bradley R, Belsky J, Crosnoe R, et al. Identifying risk for obesity in early childhood. Pediatrics 2006;118:e594-601.
12 Centers for Disease Control and Prevention. BMI for Children and Teens. Atlanta, GA: Centers for Disease Control and Prevention, 2003. www.cdc.gov/nccdphp/dnpa/bmi/bmi-for-age.htm (accessed January 20, 2006).
13 Choudhary AK, Donnelly LF, Racadio JM, Strife JL. Diseases associated with childhood obesity. AJR Am J Roentgenol 2007;188:1118-1130.
14 Guzzaloni G, Grugni G, Minocci A, Moro D, Morabito F. Liver steatosis in juvenile obesity: correlations with lipid profile, hepatic biochemical parameters and glycemic and insulinemic responses to an oral glucose tolerance test. Int J Obes Relat Metab Discord 2000;24:772-776.
15 Sagi R, Reif S, Neuman G, Webb M, Phillip M, Shalitin S. Nonalcoholic fatty liver disease in overweight children and adolescents. Acta Paediatr 2007;96:1209-1213.
16 Franzese A, Vajro P, Argenziano A, Puzziello A, Iannucci MP, Saviano MC, et al. Liver involvement in obese children. Ultrasonography and liver enzyme levels at diagnosis and during follow-up in an Italian population. Dig Dis Sci 1997;42:1428-1432.
17 Kulaga Z, Litwin M, Zajaczkowska MM, Wasilewska A, Tkaczyk M, Gurzkowska B, et al. Regionalne różnice parametrów antropometrycznych oraz ciśnienia tętniczego uczniów w wieku 7-18 lat. Probl Hig Epidemiol 2009;90:32-41.
18 Ogden CL, Carroll MD, Curtin LR, Lamb MM, Flegal KM. Prevalence of high body mass index in US children and adolescents, 2007-2008. JAMA 2010;303:242-249.
19 Santoro N, Kursawe R, D'Adamo E, Dykas DJ, Zhang CK, Bale AE, et. al. A common variant in the patatin-like phospholipase 3 gene (PNPLA3) is associated with fatty liver disease in obese children and adolescents. Hepatology 2010;52:1281-1290.
20 Lin YC, Chang PF, Hu FC, Yang WS, Chang MH, Ni YH. A common variant in the PNPLA3 gene is a risk factor for non-alcoholic fatty liver disease in obese Taiwanese children. J Pediatr 2011;158:740-744.
21 World Health Organization, 2010. http://www.who.int/mediacentre/factsheets/fs311/en/index.html (accessed March 22, 2011)
22 Feldstein AE, Charatcharoenwitthaya P, Treeprasertsuk S, Benson JT, Enders FB, Angulo P. The natural history of non-alcoholic fatty liver disease in children: a follow-up study for up to 20 years. Gut 2009;58:1538-1544.
23 Molleston JP, White F, Teckman J, Fitzgerald JF. Obese children with steatohepatitis can develop cirrhosis in childhood. Am J Gastroenterol 2002;97:2460-2462.
24 Clark JM, Diehl AM. Nonalcoholic fatty liver disease: an underrecognized cause of cryptogenic cirrhosis. JAMA 2003;289:3000-3004.
25 Brunt EM. Nonalcoholic steatohepatitis: definition and pathology. Semin Liver Dis 2001;21:3-16.
26 Baldridge AD, Perez-Atayde AR, Graeme-Cook F, Higgins L, Lavine JE. Idiopathic steatohepatitis in childhood: a multicenter retrospective study. J Pediatr 1995;127:700-4.
27 Brunt EM. Do you see what I see? The role of quality histopathology in scientific study. Hepatology 2008;47:771-774.
28 Bohte AE, van Werven JR, Bipat S, Stoker J. The diagnostic accuracy of US, CT, MRI and 1H-MRS for the evaluation of hepatic steatosis compared with liver biopsy: a meta-analysis. Eur Radiol 2011;21:87-97.
29 Yamamoto T, Tanaka H, Kobayashi H, Okamura K, Tanaka T, Emoto Y, et al. Retrospective review of Japanese sudden unexpected death in infancy: the importance of metabolic autopsy and expanded newborn screening. Mol Genet Metab 2011;102:399-406.

Received November 21, 2011
Accepted after revision March 15, 2012