CASE REPORTS

Mitochondrial Alterations in Nonalcoholic Fatty Liver Disease. Pediatric Case Description of Three Submitted Sequential Biopsies

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Abstract

Nonalcoholic fatty liver disease (NAFLD) is a clinical-pathological syndrome that encompasses a wide spectrum of morphologic alterations, ranging from simple hepatic steatosis to a more severe stage, known as nonalcoholic steatohepatitis (NASH). The purpose of this clinical report was to contribute to the understanding of mitochondrial alterations in NAFLD. The child (13-month-old) underwent initial biopsy in the year 2000 and was diagnosed with diffuse macro and microvesicular steatosis. Two additional biopsies were performed in 2001 and 2004. A high percentage of microvesicular steatosis was observed in the biopsies performed in 2000 and 2001. Mitochondrial size was slightly increased in the biopsy performed in the year 2000, significantly increased in 2001 and decreased in 2004. The presence of "mitochondrial hypertrophy" in the hepatocytes of an asymptomatic pediatric patient whose disease presentation was typical of NAFLD, excluding other pathological processes, allowed us to suspect that such a defect was considered the primary mitochondrial disorder.

Keywords

NAFLD – microvesicular steatosis – mitochondrial alterations – liver.

Introduction

Nonalcoholic fatty liver disease (NAFLD) is a clinical-pathological syndrome that includes a wide spectrum of morphologic alterations, ranging from simple lipid accumulation within hepatocytes (steatosis) to a more severe stage, known as nonalcoholic steatohepatitis (NASH), a condition that may progress to fibrosis, cirrhosis and liver failure [1, 2]. Adult NAFLD is associated with central adiposity, obesity, insulin resistance, metabolic syndrome and type 2 diabetes (Table I). Nonalcoholic hepatic steatosis is present in one-third of NAFLD cases [3].

Childhood NAFLD was first reported in the'80s [4] and is characterized by the absence of inborn errors of metabolism [5]. NAFLD, mainly the macrovesicular form, along with malnutrition are known causes of childhood steatosis. The microvesicular form may be associated with some clinical conditions separately, e.g. Reye's Syndrome, Jamaican vomiting sickness and congenital beta-oxidation deficiency [6]. In recent years, several cases of childhood NAFLD have been described. NAFLD in children has the following major characteristics: a higher prevalence in male children, a more significant increase in ALT compared to AST, high triglyceride levels and abdominal pain (usually the first reason for seeking clinical evaluation) [5, 7, 8]. Although the mechanisms that mediate this pathology have remained poorly understood, it is believed that a common factor occurs in all these disorders, involving some decrease in hepatic mitochondrial function. Childhood liver diseases, such as microvesicular steatosis, are associated with changes in mitochondria [9, 10] and an increase in the number of these metabolic organelles [10, 11]. There is a correlation with defects in the respiratory chain and thus oxidative phosphorylation [12, 13]. When this type of alteration occurs, reactive oxygen species (ROS) are generated, electrons are transferred to oxygen molecules, producing superoxide anions and hydrogen peroxide [14]. In addition, due to stagnation of mitochondrial oxidative capacity, fatty acids accumulate in the cytosol, reasserting a close relationship between steatosis and mitochondriopathy. The purpose of this clinical report was to contribute to the understanding of mitochondrial alterations in NAFLD, culminating in lipid deposition in hepatocytes.

Case Report

A periodic health examination detected an "enlarged liver" in a 13-month-old patient who was referred to

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Table I. Common causes associated with hepatic steatosis in the pediatric group (Adapted from Marion et al, 2004) [5]

General/nutritional	Acute systemic disease
	Obesity
	Insulin resistance
	Acute starvation/jejunoileal bypass/ gastric bypass
	Protein calorie malnutrition
	Inflammatory bowel disease
	Coeliac disease
	Mauriac syndrome
Infections	Hepatitis C
Metabolic	Cystic fibrosis
	Wilson's disease
	α-antitrypsin deficiency
	Fructosaemia
	Galactosaemia
	Cholesterol ester storage (Wolman) disease
	Glycogen storage disease
	Mitochondrial and peroxisomal defects of acid oxidation
	Lipodystrophies
	Abetalipoproteinaemia
	Weber-Christian disease
	Schwachman syndrome
Drug toxicity	Amiodarone
	Methotrexate
	Prednisolone
	L-asparaginase, methorexate
	Vitamin A
	Valproate
	Tamoxifen
	Zidovudine and anti-HIV treatments

the UNESP Clinical Hospital (Botucatu, SP) for further investigation. According to the parents, the child was completely asymptomatic. Feeding habits were normal, there was a negative history of contact with chemical and/or toxic products and fecal flow was regular. Ultrasonography revealed that hepatomegaly was suggestive of hepatic steatosis (Fig. 1). Apart from alkaline phosphatase (AP), liver enzyme levels were normal, as observed in Table II.

The patient was asymptomatic, despite having persistent hepatomegaly and underwent an initial biopsy in the year 2000. A diagnosis of diffuse macro and microvesicular steatosis was made. Follow-up of the patient continued for 5 years to investigate possible causes of steatosis. All other known causes of steatosis were excluded and two additional biopsies were performed during that same time period (2001 and 2004), verifying an eventual progression to fibrosis. Liver fragments were obtained by three percutaneous liver biopsies and prepared for observation under light (LM) and

Table II. Hepatic enzyme values and morphometric parameters

Year	2000	2001	2004
% microvesicular steatosis	48%	59%	42%
Total intensity of steatosis	4	4	2
ALT (U/l)	25	26	48
AST (U/l)	25	22	47
GGT (U/l)	37	50	20
AP (U/l)	460	206	215
Total cholesterol (mg/dl)	146	156	-
Triglycerides (mg/dl)	51	54	34
LDL (mg/dl)	77	-	-
HDL (mg/dl)	59	-	64
Total protein (g/dl)	6,8	6,9	7,4
Albumin (g/dl)	4,7	4,5	3,98
Glucose (mg/dl)	104	-	-
Weight (g)	9240	11165	18200
Height (cm)	72.5	82.5	107

ALT – Alanine aminotransferase; AST – Aspartate aminotransferase; GGT – Gamma-glutamyl transpeptidase; AP – Alkaline phosphatase.

transmission electronomicroscopy (TEM). The procedures were always performed using a Menghini-type liver biopsy suction needle (Hepafix), which was inserted into the mid-axillary line, at the 9th or 10th right intercostal space. The patient remained hospitalized for 24 hours. No problems occurred during or after the procedures. In both biopsies, diagnoses were similar to that of the first biopsy. However, in the last examination steatosis was less severe. During this period, no treatment had been given to the patient that could justify a decrease in hepatic steatosis.

For LM observations, the 5 mm-sections were stained with Hematoxilin-Eosin and two parameters were half-quantitatively analyzed: a) total intensity of steatosis (macro and microvesicular), classified from [1] to [4]; and b) the percentage of hepatocytes affected by microvesicular steatosis.

In TEM, for each patient, a liver tissue block was analyzed to evaluate mitochondrial area (determine mitochondrial area in 10 fields with a magnification of x6.000), and average mitochondrial density (count of existing mitochondria in 10 hepatocytes). The results obtained were compared to the average value observed in a control group of patients (children younger than 12 years with a normal liver biopsy, n=10).

In both LM and TEM morphometric analyses, the fields and/or cells were randomly selected in a standardized fashion for each biopsy, thus disabling method interference in the results obtained.

In Table II, a high percentage of microvesicular steatosis was observed in the biopsies performed in 2000 and 2001 (Figs. 2B, 2D), showing a reduction in 2004 (Fig. 2F). The intensity of total steatosis and the percentage of microvesicular stetatosis also decreased. A similar profile was observed in Fig. 3, where mitochondria were slightly

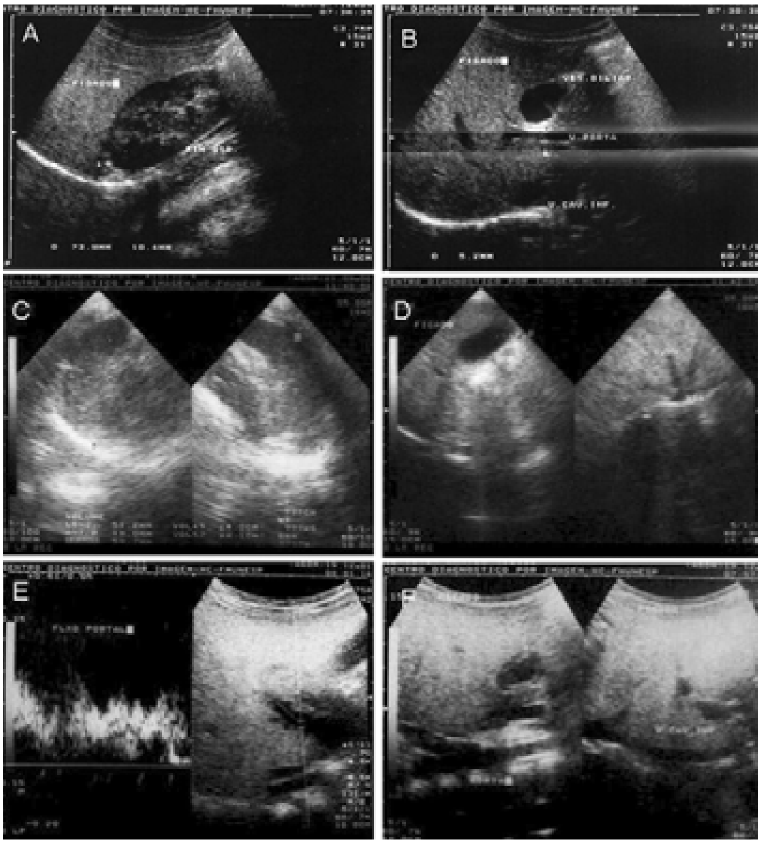

Fig 1. Ultrasonography carried out in the years 2000, 2001 and 2004. A and B in 2001: diagnosis of hepatomegaly associated with a diffuse increase in echogenicity of the liver parenchyma. C and D: diagnosis of homogeneous hepatomegaly. E and F: in 2004, diagnosis of mild hepatic steatosis.

increased in size in the biopsy performed in 2000 (Fig. 2A). Mitochondria significantly increased in size in 2001 (Fig. 2C) and decreased (values were similar to those of control) in 2004 (Fig. 2E). Regarding mitochondrial density, no significant difference was observed in the results of biopsies performed in the three time periods, as well as between the average values in the control group. The average mitochondrial densities in the biopsies performed in 2000, 2001 and 2004 were 53, 46 and 59 mitochondria/cell, respectively, and the average number was 74 mitochondria/cell in the control group.

Discussion

Based on the histological findings and laboratory results, our patient was diagnosed with NAFLD, despite being asymptomatic. According to Harrison and Bisceglie [15], many patients with NAFLD are asymptomatic, showing slightly modified serum aminotransferase levels (rarely exceeding three times the limit value). In the differential diagnosis with other liver diseases, it is important that the AST/ALT ratio be lower than 1 [16]. Alkaline phosphatase (AF) may sometimes be increased, but it usually does not exceed twice the upper limit of normal. In this particular case, no alteration was found in serum aminotransferase levels (ALT and AST), the AF value was the only change observed.

Morphological analysis of the biopsies performed demonstrated steatosis at all times, ranging from type 4 (>75%) in 2000 and 2001 to type 2 (25-50%) in 2004, and the percentage of microvesicular steatosis was 48, 59 and 42, respectively. Similar to many studies [9, 10, 17, 18] in this case we were able to demonstrate ultrastructural mitocondrial alterations within hepatocytes in the three biopsies performed. However, in all biopsies performed we observed an evident increase in mitochondrial area, called "mitochondrial hypertrophy", with a proportional increase in volume. Some authors [9, 13, 19, 20] consider that an increase in mitochondrial density which confers oncocytic appearance to the hepatocyte is a major characteristic of microvesicular steatosis in NAFLD. In our study, we did not observe this aspect in any of the biopsies performed.

In several reports and revisions [6, 21-2], the authors found that microvesicular steatosis was associated with a more severe form of NAFLD. We agree with these authors, since it has not been proven that mitochondrial damage occurs in macrovesicular steatosis.

The mechanisms that lead to macrovesicular steatosis can be related to an increased mobilization of fatty acids to the adipose tissue, synthesis of fatty acids by the liver,

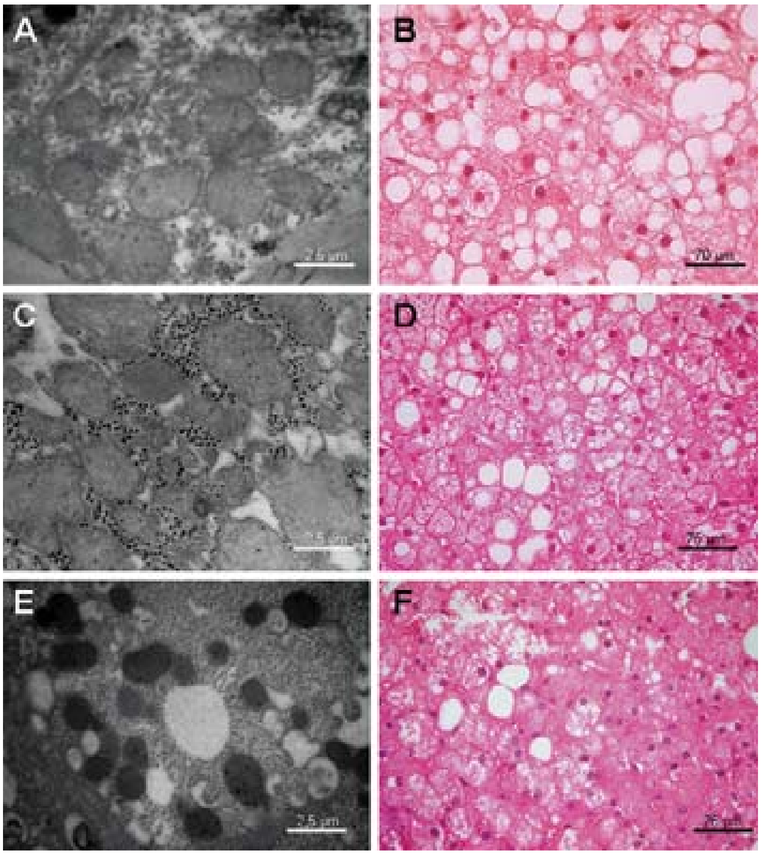

Fig 2. Light micrograph and transmission electron micrograph in different biopsies. A and B: biopsy obtained in 2000. C and D: biopsy obtained in 2001. E and F: biopsy obtained in 2004. B, D and F: Hematoxilin - Eosin staining.

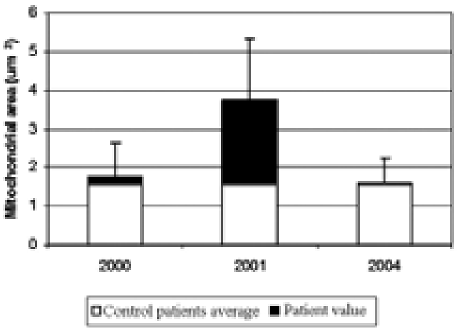

Fig 3. Mitochondrial area in hepatocytes in different biopsies. Values are expressed in square micrometers (μm^2).

esterification of fatty acids to triglycerides and diminished export of fatty acids by the hepatocyte [24]. Regarding microvesicular steatosis, defects in mitochondrial-β-oxidation may directly result in accumulation of fatty acids in the cytosol [21, 25] which can eventually increase mitochondrial area (mitochondrial "hypertrophy").

Mitochondria have a critical role in fuel oxidation for energy production. This process is carried out by a series of enzymes, generically called the mitochondrial respiratory chain. Since fuel oxidation is essential for virtually all cells, defects in the respiratory chain may cause changes in the most diverse organs. Liver disease in children can often be related to these defects [13]. According to Treem and Sokol (26), mitochondrial hepatopathy can be classified into two categories: a) primary disorders, where a specific defect in some mitochondrial component occurs, caused by nuclear or mitochondrial genomic mutation (mtDNA), and b) secondary disorders, characterized by an alteration or mitochondrial dysfunction caused by other pathological processes. The mechanisms that induce "mitochondrial hypertrophy" remain obscure. However, impairment of the respiratory chain or other mitochondrial metabolic enzyme can lead to stagnation in β-oxidation or oxidative phosphorylation. It has been suggested that an increase in mitochondrial volume occurs. The inner mitochondrial membrane (and the cristae) as well as the intermembrane space (site related to β-oxidation and oxidative phosphorylation) consequently increase in size, in an attempt to compensate for the partially or completely interrupted metabolic processes. The presence of "mitochondrial hypertrophy" in the hepatocyte of an asymptomatic pediatric patient whose disease presentation was typical of NAFLD, excluding other pathological processes, allows us to suspect that such a defect can be considered a primary mitochondrial disorder. However, we cannot completely rule out the hypothesis that other mechanisms may be involved and that such alteration is only an adaptative process of the hepatocyte. For this purpose, a larger case study is required to analyze the pathogenesis of NAFLD.

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