CASE REPORT

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Genetic disorder in carbohydrates metabolism: hereditary fructose intolerance associated with celiac disease

DANIELA PĂCURAR[1)], GABRIELA LEŞANU[1)], IRINA DIJMĂRESCU[1)], IULIA FLORENTINA ŢINCU[1)], MIHAELA GHERGHICEANU[2)], DUMITRU ORĂŞEANU[1)]

[1)]*Department of Pediatrics, "Grigore Alexandrescu" Emergency Hospital for Children, Bucharest, Romania*

[2)]*Department of Ultrastructural Pathology, "Victor Babeş" National Institute of Research and Development in Pathology and Biomedical Sciences, Bucharest, Romania*

Abstract

Celiac disease (CD) has been associated with several genetic and immune disorders, but association between CD and hereditary fructose intolerance (HFI) is extremely rare. HFI is an autosomal recessive disease caused by catalytic deficiency of aldolase B (fructose-1,6-bisphosphate aldolase). We report the case of a 5-year-old boy suffering from CD, admitted with an initial diagnosis of Reye's-like syndrome. He presented with episodic unconsciousness, seizures, hypoglycemia, hepatomegaly and abnormal liver function. The patient has been on an exclusion diet for three years, but he still had symptoms: stunting, hepatomegaly, high transaminases, but tissue transglutaminase antibodies were negative. Liver biopsy showed hepatic steatosis and mitochondrial damage. The dietary history showed an aversion to fruits, vegetables and sweet-tasting foods. The fructose tolerance test was positive, revealing the diagnostic of hereditary fructose intolerance. Appropriate dietary management and precautions were recommended. The patient has been symptom-free and exhibited normal growth and development until 10 years of age.

Keywords: celiac disease, hereditary fructose intolerance, liver biopsy.

Introduction

Celiac disease (CD) is an immune-mediated systemic disorder elicited by gluten and related prolamines in genetically susceptible individuals (mainly HLA – human leukocyte antigen), characterized by a combination of gluten dependent clinical manifestations, anti-tissue transglutaminase antibodies (TG2) and enteropathy. The disease presents with a very wide range of gastrointestinal and extra-intestinal signs and symptoms [1]. The clinical and histological pattern disappears after initiating gluten-free diet, as well as intestinal atrophy, which reverts to normal [2].

Hereditary fructose intolerance (HFI) is a rare autosomal recessive genetic disorder characterized by the absence of the enzyme called aldolase B, needed to metabolize fructose [3–5]. Untreated HFI presents with severe metabolic disturbances (hypoglycemia, lactic acidemia), renal and hepatic failure, which arise after dietary exposure to fructose, sucrose or sorbitol. If the disease is identified and treated before permanent organ injury occurs, individuals with HFI can experience a normal quality of life and they can have a long life expectancy [6].

CD has been associated with several genetic and immune disorders, but association between CD and HFI is extremely rare [7]. The prevalence of CD in the studied HFI population is 10.52% [8], similar to that of other CD-associated disorders, but CD may be silent and remain undiagnosed for years and only the genetic tests can establish the diagnosis. On the other hand, HFI incidence in cohorts of patients with CD is even lower (1/1865 adult patients). In patients with both HFI and CD, dietetic restrictions and management are a very important requirement [9].

Unfavorable evolution of the CD after gluten-free diet has been instated or of the HFI patients on a fructose-free diet presents with failure to thrive, hepatomegaly, abnormal liver function tests, renal involvement or neurological findings. In children, this is a strong argument for considering some additional food intolerance tests, genetic tests or histological examinations [10].

We present the case of a 5-year-old male patient with an association of two distinct genetic gastrointestinal disorders, HFI and CD, who after ingesting a large quantity of fructose has developed Reye's syndrome. We report this case with the consent of the family and after obtaining the approval from the Ethics Committee of the Hospital the patient was admitted to.

The aim of this paper is to draw attention to the possible rare association of genetic disorders of carbohydrate metabolism, which should be suspected especially in patients with unfavorable evolution in spite of previous diet restrictions for a metabolic intolerance. An association between CD and HFI, two distinct genetic gastrointestinal disorders, is important because poor response to the gluten-free diet as treatment of celiac disease in children calls for careful evaluation for HFI. An early diagnosis of HFI could lead to avoidance of neurological and renal involvement [11].

Case presentation

We admitted a 5-year-old boy known with celiac disease, in the Intensive Care Unit (ICU) of "Grigore Alexandrescu" Emergency Children's Hospital, Bucharest,

Romania. The initial diagnosis was Reye's syndrome. He had coma of unknown etiology. Symptoms occurred during an acute superior respiratory tract illness for which he was treated with oral anti-inflammatories. Personal history revealed CD diagnosed two years prior based on clinical criteria (poor nutritional status, distended abdomen, hepatomegaly) and laboratory tests (elevated transaminases, elevated tissue transglutaminase antibodies [>10×UNL (upper normal limit) and endomysial antibodies]. Genetic testing was positive for human histocompatibility antigens HLA DQ2/DQ8. We did not perform intestinal biopsies at the time due to parental refusal. Soon after CD was diagnosed, he was put on gluten-free diet with no improvement – growth retardation and persistent elevated transaminases, but negative tissue transglutaminase antibodies. On admission, the child had severe alteration of mental status and was ill apparent, he was reactive to pain, he presented with low weight and height for his age (7^{th} percentile weight, and 10^{th} percentile height), hepatomegaly without splenomegaly, oliguria <1 mL/kg/h and respiratory distress.

Laboratory findings showed very high transaminases values (>8×UNL), hypoglycemia (56 mg/dL), high kidney function tests and hyperammonemia. Inflammatory markers and hematological values were normal. Abdominal ultrasound revealed an increased size of the liver, diffuse liver steatosis with no abnormalities regarding the portal-splenic vascular system, as well as a diffuse renal structure alteration. The neurological status of the patient was improving after 24 hours of supportive therapy, but hepatomegaly and abnormal liver function tests were persistent during the first year of follow-up.

Reviewing the dietary history intake, we noticed that the gluten exclusion was very strict, but the child refused to eat certain fruits, vegetables and sweets, presenting episodic vomiting after ingestion of small amounts of these foods.

We performed a liver biopsy with a Braun Hepatic Luer lock set (Φ 18 G/1.2 mm) and we obtained a piece of hepatic tissue of 7/0.9/1 mm.

The histopathological findings examined by light microscopy on Hematoxylin–Eosin (HE) and Toluidine blue stainings showed non-specific micro-vascular steatosis (multiple lipid inclusions in the hepatocytes, fat deposits in the periphery of the hepatic lobules, without intralobular fibrosis or inflammation) (Figure 1). Toluidine blue staining is usually used prior to the electron microscopy examination in order to establish the orientation of the specimen.

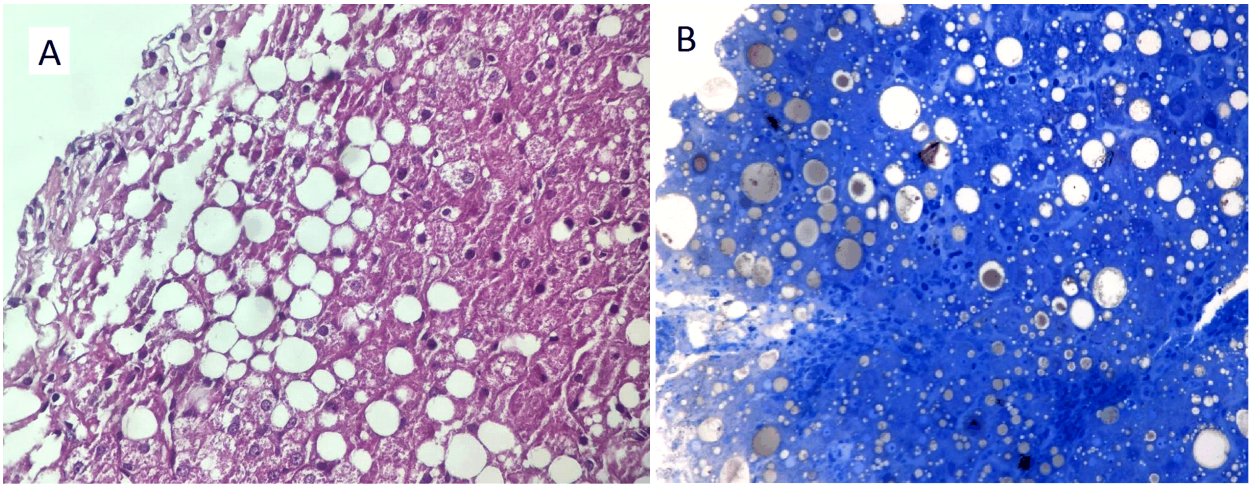

Figure 1 – ***Light microscopy (×40): (A) HE staining; (B) Toluidine blue staining. Non-specific micro-vascular steatosis (multiple lipid inclusions in the hepatocytes, fat deposits in the periphery of the hepatic lobules, without intralobular fibrosis or inflammation).***

Transmission electron microscopy was performed on small (1-mm^3) liver tissue fragments processed according to a routine Epon embedding procedure. Briefly, the tissue biopsy was fixed by immersion for four hours in 4% glutaraldehyde, cut into small pieces and refixed for one hour in 1% OsO_4 (osmium tetroxide) with 1.5% $K_4Fe(CN)_6$ (potassium ferrocyanide-reduced osmium) in 0.1 M cacodylate buffer. Afterwards, the samples were dehydrated and embedded in Agar 100 (Epon-like resin) at 60^0C for 48 hours. Routine 60 nm ultrathin sections were cut with a Leica ultramicrotome and mounted on Formvar-coated grids, stained with 1% uranyl acetate and Reynolds's lead citrate and examined with a Morgagni 268 transmission microscope (FEI Company, Eindhoven, The Netherlands), at 80 kV. Digital electron micrographs were acquired with a MegaView III CCD (charge-coupled device) and iTEM-SIS software (Olympus, Soft Imaging System GmbH, Münster, Germany). This examination revealed hepatocytes filled with lipid vacuoles and abundant glycogen granules, multiple Ito cells, some hepatocytes having mitochondria variable in size and shape, with granular matrix and fragmented cristae, peroxisomes and normal lysosomes. Areas of cytoplasmic damage were noted, known as "fructose holes", with abundant glycogen, lipid droplets, myelin figures, various areas of focal cytoplasmic degradation and large glycogen-containing vacuoles (Figure 2).

Using polymerase chain reaction (PCR) to specifically amplify exons 2–9 of human aldolase B gene and peripheral exons/introns, it was established that the patient was homozygous for codon 174 GCC mutations (alanine) to GAC (aspartic acid), known as A174D (HFI).

The patient was initiated on a fructose- and gluten-free diet and follow-up visits showed good catch-up growth after two years and a complete recovery of the liver disease.

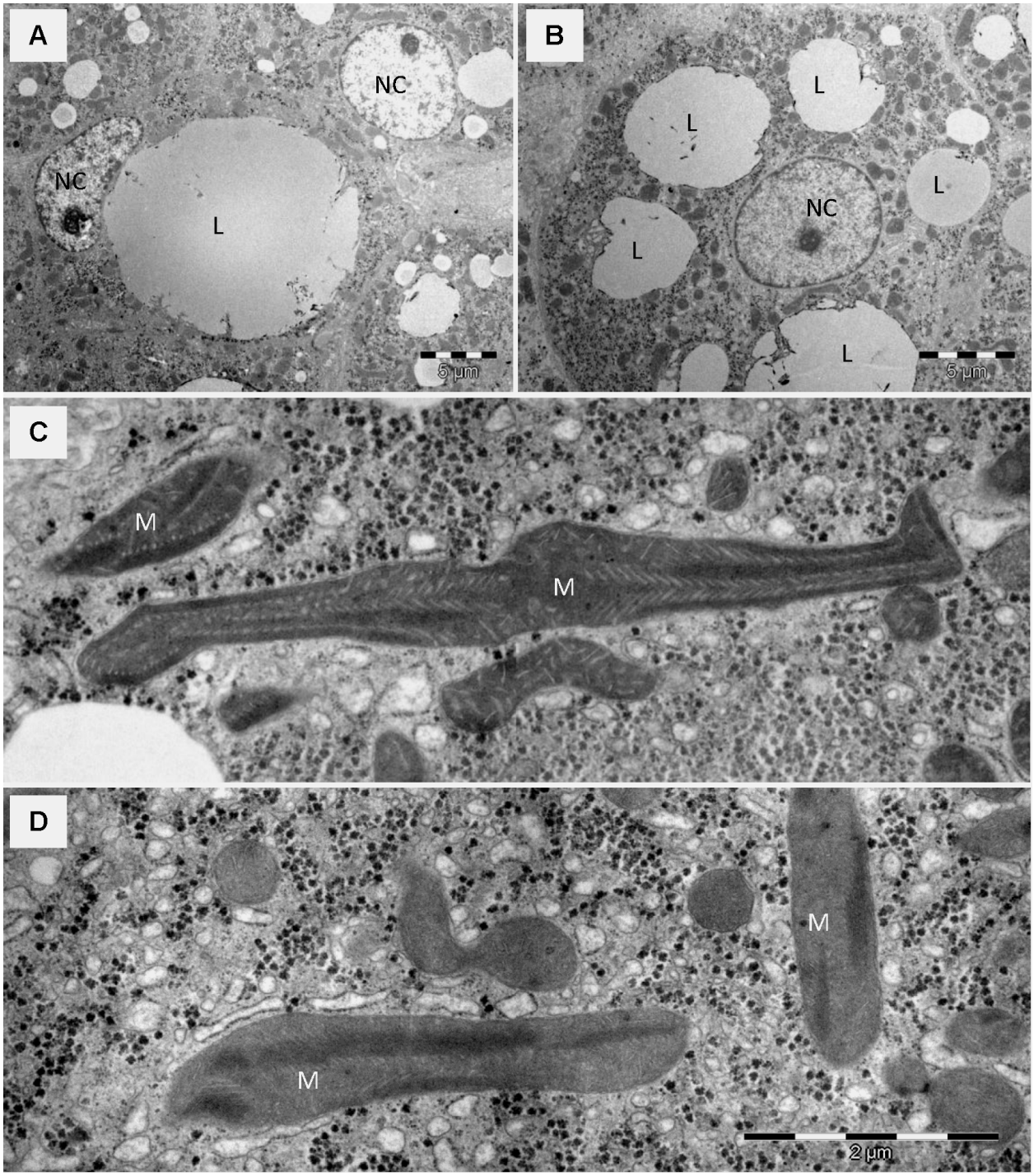

Figure 2 – ***Electron microscopy: hepatocytes filled with lipid vacuoles and abundant glycogen granules, multiple Ito cells, some hepatocytes having mitochondria variable in size and shape, with granular matrix and fragmented cristae, peroxisomes and normal lysosomes; areas of cytoplasmic damage (known as "fructose holes"), with abundant glycogen, lipid droplets, myelin figures, various areas of focal cytoplasmic degradation and large glycogen-containing vacuoles.***

Discussion

Celiac disease is a complex disorder characterized by intestinal inflammation and villous atrophy caused by intolerance to ingested gluten and related proteins in barley and rye, with many gastrointestinal and extra-intestinal signs and symptoms and a complete clinical, immunological and histological remission after initiating gluten-free diet [2].

"Fructose intolerance" is a term that defines two types of disorders. One is "hereditary fructose intolerance" and the other one is "acquired" [12]. Acquired fructose intolerance is a functional problem in the small intestine where the ability to absorb fructose is decreased [13]. HFI is a rare autosomal recessive genetic disorder (the incidence of HFI is between 1/10 000 to 1/100 000 newborns and varies in different ethnic group) characterized by the liver lack of the enzyme called aldolase B, needed to metabolize fructose [3, 5, 14]. The disease is characterized by gastro-intestinal symptoms (gas, bloating, belching, nausea, vomiting, abdominal pain, diarrhea, chronic growth restriction/failure to thrive, enlarged liver) or mimics Reye's

syndrome and metabolic disturbance: hypoglycemia, lactic acidemia, high transaminases. The symptoms occur after the child starts eating food or formula, fruits and other foods that contain fructose or sucrose [15].

As it was reported for our patient, the symptoms of gluten exposure may be similar to those of fructose intolerance in matter of liver dysfunction, but neurological manifestations are not likely in CD. Unfavorable evolution as failure of growth catch-up, hepatomegaly, persistent cytolysis syndrome and hepatic steatosis in this peculiar case of CD receiving gluten exclusion diet were the final arguments for considering some additional food intolerance.

On the other hand, the clinical onset with neurological signs suggesting Reye's syndrome may also be identified in many metabolic conditions, imposing a large differential diagnosis [16–18].

To the best of our knowledge, there are only a few cases reporting the association between CD and HFI. One study conducted in Italy, which included patients with HFI, who were genotyped for the human leukocyte antigen (HLA)-II CD associated alleles, aimed to identify patients bearing the risk DQ2/DQ8 molecules encoded by HLA haplotypes (DQA1*05/DQB1*02/DQA1*03/DQB1*0302) and to classify patients into risk classes: most HFI patients belonged to the low-risk class (G5), only three of 20 belonged to the high-risk class (G2) [19].

The prevalence of CD in the studied HFI population (four females out of 38 patients; 10.52%) was higher or similar to that of other CD-associated disorders: *i.e.*, 8% for type 2 diabetes, 5–12% for Down syndrome, 4.1–8.1% for Turner's syndrome, 8.2% for Williams syndrome, and 1.7–7.7% for immunoglobulin A (IgA) deficiency [3, 20].

The possibility of an association between HFI and CD was investigated further by the Italian HFI patients' organization (*The Italian Association of Fructose Intolerance*), through an anonymous questionnaire addressed to HFI patients (or their guardians). Globally, the incidence of CD among Italian HFI patients was 10.52%, which is significantly higher than the frequency estimated for the general population (1–3%) [2, 8]. In addition, three of the four HFI patients who are CD-positive had high transaminases, short stature and low weight, which suggests that these signs could serve as a guide for CD diagnosis, at least in this subgroup of patients. Accordingly, up to 9% of adults with increased transaminases levels of uncertain cause have been reported to have silent CD [21].

On the other hand, we should not exclude the possibility that some HFI patients who were not tested for CD may have the disease, considering that it may be silent and remain undiagnosed for years or for the patient's entire life.

In the same study, there was only one HFI patient identified among the 1865 adult patients with CD from Southern Italy, who are currently being monitored in the tertiary clinic [8]. Thus, the history may be vital for the presented case in order to reach the complex diagnosis. It is very important to take an extensive dietary history and ask about food rejection, especially in children who refuse all sweets after becoming ill early in life [22, 23].

The unfavorable evolution of the liver disease and the dietary history shed some light on the association of CD and HFI. The leukocyte molecular analysis of the gene on chromosome 9 may provide definitive evidence of a mutation of human aldolase B gene at the q22.3 band. The genetic mutation in the presented case was homozygous for codon 174 GCC (alanine) to GAC (aspartic acid), known as A174D, specific for HFI [24]. As of 2010, more than 20 mutations have been reported on the gene *ALDOB* [Online Mendelian Inheritance in Man (OMIM) *612724], located on chromosome 9q22.3, most of them single-base substitutions, the most prevalent being p.A149P, pA147D, N334k. These are responsible for more than 84% of *ALDOB* mutations in HFI cases worldwide [5, 25–27].

In many studies, liver biopsy is not recommended as a diagnostic procedure, except for cases of certain liver disease. HFI patients develop non-alcoholic fatty acid liver disease and fibrosis [28].

Histological findings in a liver biopsy specimen from an untreated patient may show evidence of hepatocellular involvement, including areas of focal necrosis, fatty degeneration in peripheral lobules, bile duct proliferation, and late changes of portal and biliary cirrhosis [9], histological changes also shown in the presented case. Electron microscopy revealed HFI particular lesions: concentric membranous arrays in hepatocytes, areas of cytoplasmic damage, 'fructose holes' (lucent partially membrane bound areas of cytoplasm), abundant glycogen, prominent lipid droplets, large autophagic glycogen-containing vacuoles, myelin figures, focal cytoplasmic degradation [28].

In symptomatic patients, elimination of fructose from the diet may also serve as a diagnostic test, as well as a therapeutic method, because all symptoms should completely resolve.

We believe that HFI is underdiagnosed because of the wide and nonspecific spectrum of clinical signs. Patients with late or with no diagnosis can survive if they learn to reject foods that cause them discomfort [14].

This case presents an association between HFI and CD detected in a 5-year-old patient who presented with acute onset of neurological impairment, hypoglycemic coma, hepatomegaly and severe liver and kidney dysfunction. As in similar cases of HFI, it presented initially with episodic unconsciousness, seizures, hypoglycemia, hepatomegaly, and abnormal liver function and Reye's-like syndrome was considered as first. After establishing the diagnosis, appropriate dietary management was recommended and the patient has been symptom-free and exhibited normal growth and development [29].

Conclusions

Children with intolerance to carbohydrates often present with unexplained signs and symptoms. An association between HFI and CD is rare and the diagnosis of a complex genetic disorder in carbohydrate metabolism was extremely important in this case in order to obtain remission of the liver disease and also for establishing the dietetic restrictions and management. In our CD suffering patient with hepatic involvement and partial response to exclusion diet, the extensive dietary history was very important, especially the data about the onset of symptoms after the intake of sweets, fruits and vegetables. Clinical findings and specific genetic testing (identifying the specific gene mutation in the aldolase gene) confirmed the association between the CD and the HFI. In HFI, the biopsy of the liver is not recommended as a diagnostic procedure, except in cases

of certain liver disease, but the specific histological and electron microscopy findings were useful for the diagnosis. With the dietary restriction, both of gluten and fructose, the patient was symptom-free, exhibited normal growth and development and the liver function tests were normal.

Conflict of interests

The authors have no potential or actual conflict of interest, financial or any kind, regarding this publication.

Informed consent

All procedures followed were in accordance with the ethical standards of the responsible Committee on Human Experimentation and with the Helsinki Declaration of 1975 as revised in 2000, with the Ethical Local Committee acceptance. Informed consent was obtained from the patient and his parents for being included in the study.

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Corresponding author

Iulia Florentina Ţincu, MD, PhD Student, Emergency Department, "Grigore Alexandrescu" Emergency Hospital for Children, 30–32 Iancu de Hunedoara Avenue, Sector 1, 11743 Bucharest, Romania; Phone +4021–316 93 66, Fax +4021–312 79 38, e-mail: if_boian@yahoo.com

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