Original paper

Long-term follow-up in children with progressive familial intrahepatic cholestasis type 2 after partial external biliary diversion with focus on histopathological features

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Progressive familial intrahepatic cholestasis (PFIC) comprises a group of rare cholestatic liver disorders of childhood that could lead to liver cirrhosis. Nowadays, the partial biliary diversion procedure is still a therapeutic option in non-cirrhotic children with PFIC1 or PFIC2 after an ineffective ursodeoxycholic acid (UDCA) therapy. However, the relevant disadvantage of the partial external biliary diversion (PEBD) is that adolescent patients could not accept a permanent stoma. In some of them, despite of good clinical and biochemical results of this procedure, the ileal exclusion (IE) procedure had to be performed many years after PEBD.

Our aims were to find the most characteristic early microscopic features of the disease as well as to compare changes in the liver biopsy specimens at the time of diagnosis and long-time (more than 10 years) after a surgical procedure.

We examined retrospectively 8 liver biopsies from 4 PFIC2 patients comparing the results from the first biopsies done at the time of PFIC diagnosis and the second ones, done many years after PEBD.

The characteristic lobular rosette formations of hepatocytes were found in all patients at the time of diagnosis. Cholestasis was observed in each patient, but only in two of them, centrally located bile plugs were found. The majority of hepatocytes showed degenerative changes from mild to severe degree. In the follow-up biopsies, cholestasis completely disappeared in 3 patients and decreased significantly in 1 other patient. Based on Batts and Ludwig fibrosis scoring system, the liver fibrosis had resolved in two out of three patients.

The formation of lobular rosettes with centrally located bile plugs and degenerative changes of hepatocytes seem to be the most characteristic microscopic features in early liver biopsies in PFIC2 patients. Partial external biliary diversion significantly improved the clinical, anthropological, biochemical as well histological outcome of the patients.

Key words: progressive familial intrahepatic cholestasis, partial external biliary diversion, follow-up, children, liver, histopathology.

Introduction

Progressive familial intrahepatic cholestasis (PFIC) constitutes a group of rare cholestatic liver disorders; three types of the disease have been identified based on mutations in the hepatocellular transport system genes involved in the formation of bile. Among them, progressive familial intrahepatic cholestasis type 2 (PFIC2) is associated with mutations in the *ABCB11* gene, encoding the bile salt export pump (BSEP), a major exporter of primary bile acids from hepatocytes to canaliculi [1, 2, 3].

The microscopic features that might be found in liver biopsy specimens of PFIC type 1 or 2 patients are strongly heterogeneous and non-specific; they include the following: enlarged portal tracts with inflammation, ductular proliferation and fibrosis, canalicular cholestasis, absence of true ductular proliferation, periportal biliary metaplasia of hepatocytes, pronounced portal/lobular fibrosis, pronounced portal/lobular inflammation, hepatocellular necrosis, giant cell transformation and perturbed liver architecture [3, 4, 5].

Before 1990s, the liver transplantation (LTx) was the only therapeutic method for PFIC patients. Recently, two other surgical (non-transplant) procedures such as the partial biliary diversion (external or internal) and ileal exclusion (also known as ileo-ileal by-pass) were proposed for non-cirrhotic PFIC patients. What's more, we currently know that PFIC1 post-LTx patients could develop the fatty liver disease. Therefore, partial biliary diversion is still the therapy of choice in non-cirrhotic patients with low-GGT PFIC after an ineffective ursodeoxycholic acid (UDCA) therapy. However, the relevant disadvantage of the partial external biliary diversion (PEBD) is that adolescent patients could not accept a permanent stoma. In some of them, despite of good clinical and biochemical results of this procedure, the ileal exclusion (IE) procedure had to be performed many years after PEBD [6, 7, 9].

The aim of this study was to present the long-term outcomes of PEBD in a group of 4 children with genetically confirmed PFIC2 focusing on histopathological features. To the best of our knowledge, this is the first report presenting such a long-term follow-up. We also tried to find the most specific histopathological features found at the time of diagnosis (an early stage of the disease) as articles concerning the microscopic features are also sparse.

Material and methods

This is a long-term observational, single-centre study of patients with PFIC2. Over 30 years, there was a number of 44 patients diagnosed with PFIC2. Finally, only 4 patients were recruited to the study. The inclusion criteria were as the following:

1. The diagnosis of PFIC2 was confirmed by anamnesis (history of jaundice, and/or pruritus), clinical and biochemical abnormalities (hepatomegaly, elevated bile acids concentration in serum with normal level of GGTP) and subsequent molecular analysis (done at the last follow-up visit).
2. Patient underwent a partial external biliary diversion (PEBD) procedure.
3. Before the final decision of closing the stoma, the liver biopsy was performed (many years after PEBD).

A retrospective chart review of patients' medical records concerning the anthropometry data (weight, height), as well biochemical (total serum bilirubin, serum transaminases and gamma-glutamyl transpeptidase, serum bile acids), histopathological (microscopic examination of liver biopsies) and molecular (*ABCB11* gene mutations) were collected.

The liver biopsy specimens, between 1.0 and 1.5 cm in length, were fixed in 4% formalin and stained by hematoxillin and eosin, PAS method (periodic acid + Schiff reagent) and PAS method after diastase digestion, Azan method and reticulin impregnation. Additionally, the immunohistochemical examination by the use of cytokeratins CK7 and CK19 (Dako) was performed to demonstrate or exclude the ductular proliferation. To assess the histological activity of microscopical changes, the following categories of lesions were considered retrospectively: presence of inflammatory infiltrates (grading according to Batts and Ludwig classification) in the portal spaces and lobules with or without piecemeal necrosis, degree of fibrosis (grading according to Batts and Ludwig classification), lobular disarray, rosette formations, proliferation of bile ducts and ductules with or without ductitis, lobular necrosis, hepatocyte giant cell transformation, steatosis and degenerative changes in the hepatocytes, canalicular bile plugs, cholestasis in the hepatocytes and in bile ducts. For the purpose of the present study there was created the scoring system based on complex microscopical changes: cholestasis (minimal, mild, severe), rosette formation (present, absent), inflammation and fibrosis staged according to five-degree Batts and Ludwig score (0-4), piecemeal necrosis (present, absent), proliferation of bile ducts (present, absent), steatosis (none, mild, moderate, severe).

Results

The study presents longitudinal clinical, anthropological, laboratory and histopathological data of 4 PFIC2 patients. The range of follow-up period was 10-15 years. Individual patients' characteristics is summarized in Table I.

All patients experienced a sustained improvement of pruritus after PEBD procedure with normalization of serum bilirubin and bile acids levels as well

Table I. Individual patients' characteristics and antropometry and laboratory data

Patient	1	2	3	4
Gender	Male	Male	Female	Female
Molecular variant and protein effect	Heterozygote c.890 A>G, p.Glu297Gly, no identified mutation on the 2nd allel	Heterozygote c.1445 A>G, p.Asp482Gly; c.2494 C>T, p.Arg832Cys	Homozygote c.1445 A>G, p.Asp482Gly	Heterozygote c.1445 A>G, p.Asp482Gly; c.890 A>G, p.Glu297Gly
Age at the 1st visit in the referral center (y – years, m – months)	2 y	2 y	12 y	1 y 2 m
The last visit before PEBD				
Age (y – years, m – months)	2 y	2 y	14 y	1 y 2 m
Weight, kg	11.5	11.9	26.4	9.2
Weight-for-age, percentile	25-50	25-50	<3	25-50
Height, cm	82.0	85.0	124.3	73.2
Height-for-age, percentile	3-10	25	<3	10-25
Laboratory data; (laboratory references)				
Total serum bilirubin, mg/dl; (< 1.0)	26.4	4.2	2.6	10.9
Serum bile acids, μmol/l; (< 10.0)	465	89	29.4	518
ALT, IU/L; (< 39)	40	45	54	48
AST, IU/L; (< 52)	n.a.	89	60	62
GGT, IU/L; (< 29)	11	6	10	27
Pruritus	severe	severe	severe	severe
Age at the moment of PEBD procedure (y – years, m – months)	2 y 4 m	2 y 1 m	14 y 2 m	1 y 5 m
The last visit before IE				
Age (y – years, m – months)	16 y 6 m	17 y	21 y 10m	16 y
Antropometry				
Weight, kg	61.0	66.0	47.7	48.0
Weight-for-age, percentile	25-50	25-50	n.a.	10-25
Height, cm	174.0	170.0	151.0	153.5
Height-for-age, percentile	25-50	10-25	n.a.	3
Laboratory data; (laboratory references)				
Total serum bilirubin, mg/dl; (< 1.0)	<1.0	<1.0	<1.0	<1.0
Serum bile acids, μmol/l; (< 10.0)	3.6	4.7	7.0	1.3
ALT, IU/L; (< 39)	15	26	28	20
AST, IU/L; (< 52)	15	25	20	17
GGT, IU/L; (< 29)	6	15	18	6
Pruritus	absent	absent	absent	absent
Age at the moment of IE procedure (y – years, m –months)	16 y 8 m	17 y 6 m	22 y	16 y 4 m

PEBD – partial external biliary diversion; IE – ileal exclusion; ALT – alanine aminotransferase; AST – aspartate aminotransferase; GGT – gamma-glutamyl transpeptidase; n.a. – not analyzed

transaminases. Both height and weight improved in PFIC2 patients after PEBD procedure. Based on presented data we conclude that this procedure is highly effective in PFIC2.

At the time of PFIC diagnosis, the characteristic lobular rosette formations of hepatocytes were found in all patients. At that time, cholestasis was observed in all patients, but only in one of them it was assessed as severe. The presence of intralobular bile plugs (Figs. 1, 2) was found in 50% of patients. The majority of hepatocytes showed degenerative changes from mild to severe degree. None patient presented with

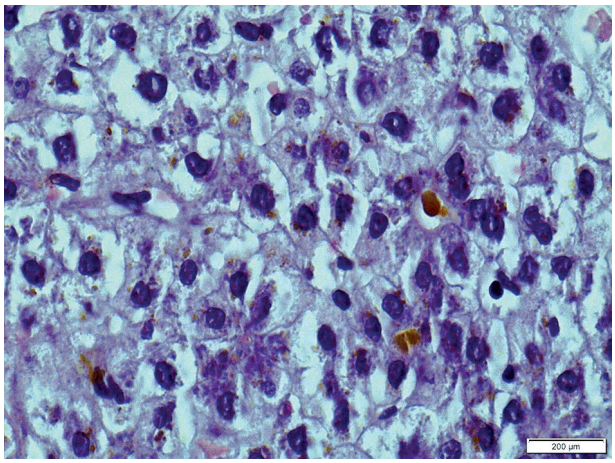

Fig. 1. Histopathological features of PFIC2. Severe intrahepatic cholestasis, rosette formation, intralobular bile plugs. Hematoxylin and eosin stain. Original magnification 200×

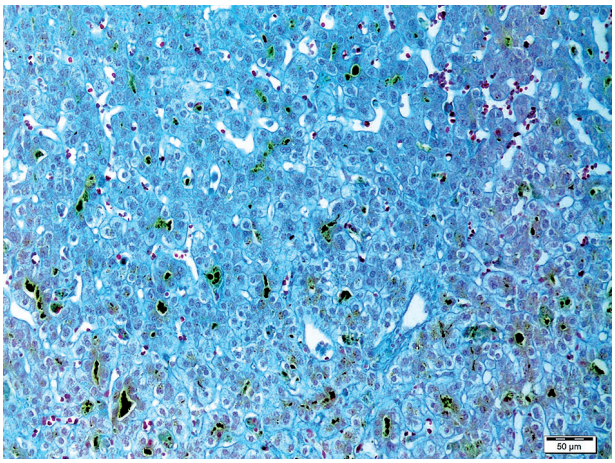

Fig. 2. Histopathological features of PFIC2. Azan stain. Severe intrahepatic cholestasis with multiple bile plugs. Original magnification 50×

Table II. Histopathological features of PFIC2 patients

Patient	1	2	3	4
Liver biopsy before BEPD				
Cholestasis	mild	mild	mild	severe
Rosette formation	+	+	+	+++
Intralobular bile plugs	+	–	–	++
Batts and Ludwig inflammation scoring system	0	0	0	0
Batts and Ludwig fibrosis scoring system	1	0	2	2
Piecemeal necrosis	–	–	–	–
Proliferation of bile ducts	–	–	–	–
Steatosis	–	–	–	–
Liver biopsy before IE				
Cholestasis	minimal	minimal	minimal	mild
Rosette formation	–	–	–	+
Intralobular bile plugs	–	–	–	–
Batts and Ludwig inflammation scoring system	0	0	0	0
Batts and Ludwig fibrosis scoring system	0	0	0	2
Piecemeal necrosis	–	–	–	+
Proliferation of bile ducts	–	–	–	–
Steatosis	Mild, macrovesicular	–	–	–

features of inflammation on liver biopsy besides liver fibrosis was observed in 3 patients' biopsies.

In the follow-up biopsies, cholestasis had completely disappeared in 3 patients and decreased significantly in 1 other patient. Intralobular bile plugs were not observed. Based on Batts and Ludwig fibrosis scoring system, the liver fibrosis had resolved in two out of three patients. Histopathological features of PFIC2 patients are presented in Table II.

Discussion

In our study we analyzed retrospectively 8 liver biopsy specimens of 4 PFIC2 children who underwent PEBD. Our aims were to find the most characteristic early microscopic features of the disease as well as to compare changes in the liver biopsy specimens at the time of diagnosis and long-time (more than 10 years) after a surgical procedure.

The microscopic features found in the liver biopsy specimens of PFIC2 patients are nonspecific and changing with time of the disease. Therefore, the final diagnosis of PFIC could be a challenge. According to our experience, the most prominent early features of PFIC2 were the lobular rosette formations with centrally located bile plugs and hepatocytes degeneration from mild to severe degree. A comprehensive study of Evanson *et al*. [10] assessing 22 liver biopsies from 12 PFIC2 patients identified similar results. All patients had hepatocellular cholestasis and most of them had canalicular bile plugs. Pseudorosette formation was present in 20 out of 22 liver biopsies.

The main role of PEBD procedure is to interrupt the bile salt recirculation, to relieve pruritus, and potentially to improve liver function and overall clinical outcome. The presented data are in concordance with those in the literature regarding the effectiveness of PEBD on clinical, biochemical and anthropological outcome of PEBD patients [11].

Most articles on PFIC concentrate on molecular findings and clinical outcomes, thus the information concerning histopathologic findings are limited, especially with such long-term follow-up.

David-Spraul *et al*. [5] analyzed the liver histology of 30 patients with either PFIC1 or PFIC2 but their observations included only biopsies at the time of diagnosis and after 1 year. The studied group was not treated with surgical methods, additionally.

The only study including patients after PEBD with long-time follow-up is paper authored by Arnell *et al.* [12]. They enrolled 18 children with PFIC1 and PFIC2. Their analysis included biopsies at the time of diagnosis, 3 and 5 years after the surgical procedure, and more than 10 years after PEBD. Authors assessed the activity of microscopic changes based on 7 parameters (inflammation, fibrosis, steatosis, giant cell transformation, cholestasis and ductular reaction). They concluded that the intensity of each feature reduced with time after PEBD procedure; therefore PEBD should be the first choice of surgical treatment in non-cirrhotic PFIC patients. We would like to emphasize that our results are in accordance with Arnell *et al*. findings.

In the presented study, PEBD procedure resulted in histopathological improvement in all PFIC2 patients. The fact that is very important is that the features of liver fibrosis had resolved in almost all PFIC2 patients.

We presented detailed analysis of microscopic features in livers of children with PFIC2 at the moment of diagnosis and more than 10 years after PEBD. The major limitations of our study are a small group of patients and an availability of liver biopsies more than 10 years after surgical procedure in 4 children only. However, the long-time follow-up of our PFIC2 patients is a significant advantage because the articles concerning the long-term follow-up are sparse in a worldwide literature. Further studies on bigger groups of children are needed.

Conclusions

The formation of lobular rosettes with centrally located bile plugs and degenerative changes of hepatocytes seem to be the most characteristic microscopic features in early liver biopsies in PFIC2 patients.

Partial external biliary diversion significantly improved the clinical, anthropological, biochemical as well histological outcome of the patients.

The authors declare no conflict of interest.

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