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Metabolic profiles and liver steatosis in children with liver transplantation for progressive familial intrahepatic cholestasis type 1

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ABSTRACT

Aims: Severe liver steatosis is common complication post-transplantation in patient with progressive familial intrahepatic cholestasis type 1 (PFIC1). We aimed to evaluate metabolic profiles in children who received orthotopic liver transplantation (OLT) for PFIC1 and their correlation with the development of fatty liver disease (FLD). Methods: Patients transplanted for PFIC1 disease were identified and matched in a 1:2 ratio with patients transplanted for other indications. Liver biopsy was used to determine the presence of FLD. Lipid profiles were compared in the 2 groups. To investigate mechanistic pathways leading to the development of FLD, volatile organic compounds (VOCs) were analyzed on prospectively collected samples using a selective ion flow tube mass spectrometry. Results: Five children with PFIC1 disease and 10 children with other indications for liver transplantation were included. Liver biopsies demonstrated that all children with PFIC1 had moderate-to-severe steatosis. The PFIC1 group had significantly lower HDL (20.9±6.0 mg/dL versus 51.3±17.1 mg/dL, respectively, $p < 0.001$) and total cholesterol levels (72±8.0 versus 156±103, $p < 0.001$) post transplantation compared to the other group. VOCs, including acetaldehyde, ammonia and pentane were elevated in the PFIC1 group, while isoprene was lower. Conclusion: Fatty liver disease was a consistent finding in the patients with PFIC1 post OLT. Lipid levels were uniformly low in PFIC1 patients. Metabolomic analysis suggested that pathways in oxidative stress, gut bacteria production and cholesterol synthesis were different in children with PFIC1.

Keywords: Liver transplantation, Steatosis, Progressive familial intrahepatic cholestasis, Children

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INTRODUCTION

Progressive familial intrahepatic cholestasis type 1 (PFIC1) is an autosomal recessive disorder that causes disruption of bile formation. It is caused by a genetic

mutation in the ATP8B1 gene [1–5]. The estimated incidence is 1 in 50,000 to 100,000 living births [1, 6, 7]. PFIC1 usually appears in the first months of life with recurrent jaundice, cholestasis and pruritus that later become persistent. Extra-hepatic manifestations are also present such as sensorineural hearing loss, short stature, diarrhea and pancreatitis [1–3, 6, 7]. These patients usually develop progressive cholestatic liver disease leading to portal hypertension, cirrhosis and liver failure. Partial external biliary diversion might be helpful at the beginning of the disease, but the majority of the patients will require orthotopic liver transplantation during childhood [1, 2, 8–10]. Severe liver steatosis is a common complication post transplantation with no clear etiology [1, 7]. Possible mechanisms include the fact that PFIC1 is a systemic disease affecting other organs including pancreas and the small intestine causing disruption in the enterohepatic circulation of bile acids [11–13]; change in the expression of a specific nuclear factor such as farnesoid X receptor (FXR) leading to alteration in the bile acid homeostasis [14–16]; and certain mutations in the PFIC1 gene can be associated with steatosis post-orthotopic liver transplantation (OLT) [17, 18]. Recently, breath testing has been used to perform metabolomic analysis of volatile organic compounds (VOCs) in breath that reflect the metabolic status of the host. By using selective ion flow tube mass spectrometry (SIFT-MS) to analyze VOCs in the breath of obese children, we have recently shown that those with non-alcoholic fatty liver disease have a unique breath signal [19].

Thus, the aims of this study were to evaluate metabolic profiles in children who received liver transplantation for progressive familial intrahepatic cholestasis type 1. We also aimed to evaluate the correlation of the metabolic profiles with hepatic steatosis and to investigate mechanistic pathways that can lead to the development of fatty liver disease (FLD).

MATERIALS AND METHODS

Patient population and clinical data

A review of children who received a liver transplantation between 2002 and 2009 at the Cleveland Clinic was done. Two groups of patients were identified. The first group is patients who were transplanted for PFIC1 (5 patients) and the second group is aged-matched patients that received a liver transplant for other liver diseases [10 patients; biliary atresia (4), congenital hepatic fibrosis (1), idiopathic cholestasis (2), primary sclerosing cholangitis (1) and Alagille syndrome (2).

Demographic, clinical and laboratory data, lipid profiles and liver biopsies were collected. Lipid profiles collected pre-OLT, 6 months, 1 year and more than two years post-OLT. The lipid profiles included the total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL) and triglycerides (TG).

The presence of FLD was assessed in liver biopsies post-OLT. Liver steatosis was graded as mild (5–33%), moderate (34–66%) or severe (more than 66%). The presence of steatohepatitis was determined by an expert pathologist. Fibrosis was staged separately according to Kleiner et al. [20].

Breath testing collection

To investigate mechanistic pathways leading to the development of FLD, breath testing for volatile organic compound (VOC) was used. This is a novel, non-invasive, unbiased tool that analyzes breath VOC by selective ion flow tube mass spectrometry (SIFT-MS). The VOC analysis can provide valuable information about the metabolic condition of an individual.

All exhaled breath samples were collected after eight hours of fasting. The exhaled breath samples underwent gas analysis using SIFT-MS on a VOICE200® SIFT-MS instrument (Syft Technologies Ltd, Christchurch, New Zealand). Data was collected by selected ion monitoring (SIM) of product ions of pre-selected compounds.

Statistical analysis

Data are presented as mean ± standard deviation, median [25th, 75th percentiles] or N (%). Univariate analysis was done to compare patients with PFIC1 post transplant versus no PFIC1 post transplant. Student's t-tests or the non-parametric Wilcoxon rank sum tests were used to compare continuous variables and Pearson's chi-square tests were used for categorical variables.

RESULTS

Patient characteristics

Fifteen patients were included in the study (5 post-OLT for PFIC1 and 10 post-OLT for other liver diseases). The clinical characteristics are summarized in Table 1. The mean age of patients with PFIC1 at the time of diagnosis was around 21 months old and there was no difference compared with the non-PFIC1 group. There was also no difference in gender or age at OLT for both groups. The average BMI percentile at one year post-OLT in the PFIC1 patients was 59.7% and it remained within normal range despite the development of steatosis. The fasting glucose levels were also normal (60–100 mg/dL) in the post transplanted patients for PFIC1. Liver biopsies of children with PFIC1 were done within two years from OLT (mean 12.8±8.3 months) and all had moderate to severe steatosis(grade 2–3). Three of the five patients with PFIC1 (60%) had steatohepatitis with hepatocyte ballooning and inflammation. More importantly two out of the three had bridging fibrosis or cirrhosis. One patient

developed ascites and portal hypertension within three years after OLT.

Table 2 gives lipid profiles and fasting glucose at different time points in the PFIC1 patients. Despite the progression to develop FLD, lipid levels remained uniformly low post–OLT. The diamond plot graph in Figure 1, where the midline represents the mean, shows that the PFIC1 group had significantly lower HDL levels post transplantation compared to the non-PFIC1 group (20.9±6.0 mg/dL versus 51.3±17.1 mg/dL, respectively, $p < 0.001$). Total cholesterol was also lower in the PFIC1 group (72±8.0 versus 156±103, $p < 0.001$) but this could be a reflection of the lower HDL levels. Furthermore, as seen in the Figure 1, LDL, VLDL and TG were also low in the PFIC1 group, however this did not reach statistical significance.

Table 1: Clinical characteristics of both groups.

	PFIC1 Disease (n=5)	Non-PFIC1 Disease* (n=10)	P Value
Mean age a diagnosis (months)	21.5 (0-96)	20.1 (0-144)	0.95
Age at OLT (years)	9.7 (2.61-13.67)	7.6 (0.57-16.91)	0.53
Gender (% female)	60	40	-
BMI at 1 year post-OLT BMI Percentile	16.96 (15-20.1) 59.7%	19.14 (15.2-23) 82.02 %	0.10
Rejection (%)	0	20	-
FLD (%)	100	0	-

* Non-PFIC1 disease group: Biliary atresia=4, AlagilleSd=2, Idiopathic cholestasis=2, PSC=1, CHF=1

Analysis of volatile organic compounds

Changes in certain VOCs were also noticed in PFIC1 patients (Figure 2). Acetaldehyde, a marker of endogenous alcohol production by the gut micro flora was markedly elevated in the PFIC1 group compare with the non-PFIC group (57.9±5.12 ppb versus 24.23±4.7 respectively). Ammonia (72.9±9.7 versus 41.8±4.7) and Pentane (18.3±4.3 versus 10.7±1.7) were also higher in the PFIC1 group. Isoprene a by-product of cholesterol synthesis was lower in this group (7.74±1.4 versus 11.25±2.2) ($p < 0.05$ for all VOCs). This is consistent with the lipid profiles in the PFIC patients (Table 2).

DISCUSSION

The main findings of our study are that: 1) all PFIC1 patients developed liver steatosis after liver transplant,

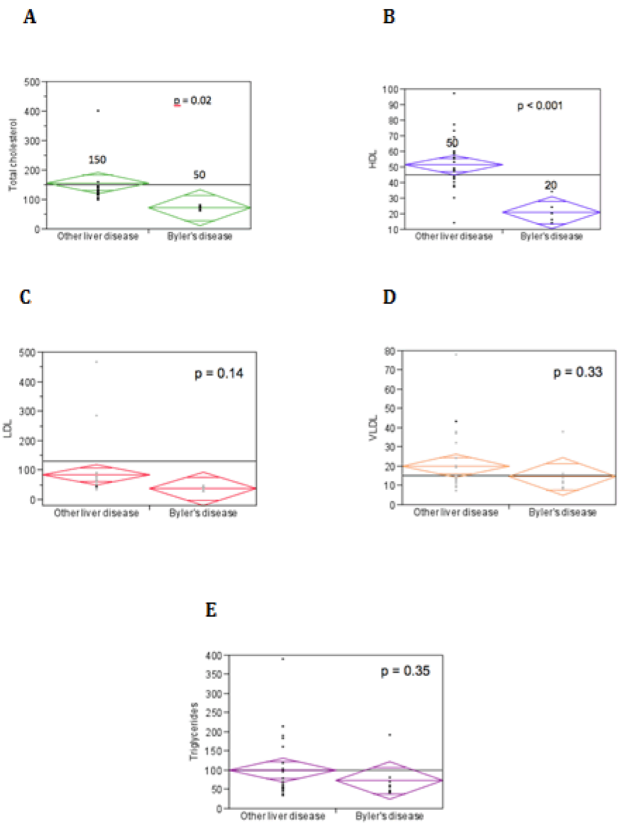

Figure 1 (A, B): Total cholesterol and HDL markedly low in the PFIC1 group with statistical significance, (C, D, E): Low density lipoprotein (LDL), very low density lipoprotein (VLDL) and triglycerides (TG) also low in the PFIC1 group, although no statistical significance was reached.

Table 2: Lipid profiles and fasting glucose in PFIC1 group at different time points

	Pre-OLT	6 months	1 year	>2 years
TC (75–169 mg/dL)	171	62	73.25	72.25
HDL (>55 mg/dL)	9	20	23.5	18.5
LDL (50–109 mg/dL)	132.7	34	36.5	37.25
VLDL (5–24 mg/dL)	29.3	8	13.25	17.5
TG (25–120 mg/dL)	146.7	40	66.5	87.75
Fasting glucose (70–113 mg/dL)	81.2	81.6	86.8	79.6

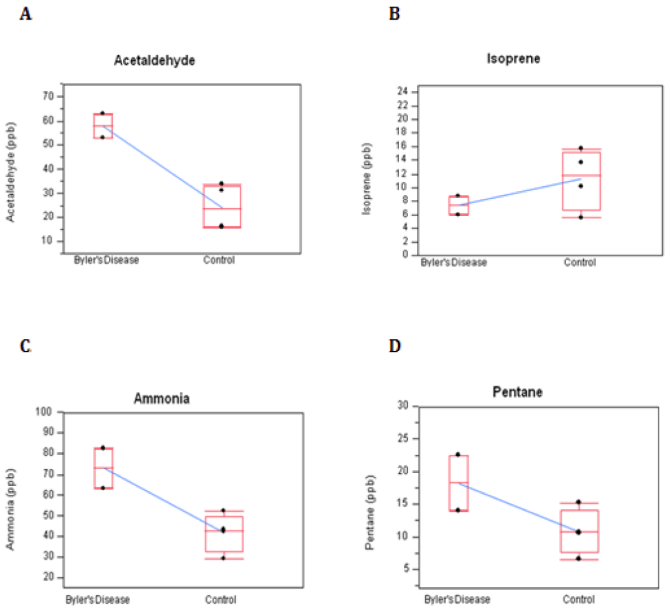

Figure 2: Analysis of volatile organic compound (VOC). Acetaldehyde, ammonia and pentane were higher in the PFIC1 group. Isoprene was lower in this group.

with 60% developing steatohepatitis and 40% progressing to advanced fibrosis indicating a significant impact on the liver graft; 2) the PFIC1 group had significantly lower HDL and TC levels post-transplantation while maintaining low BMIs, when compared to the non-PFIC1 group; 3) changes in certain VOCs, including those involved in the pathways of endogenous alcohol production, oxidative stress and cholesterol synthesis were noted in the PFIC1 patients.

The medical treatment for PFIC1 is usually unsuccessful and most patients will progress to liver failure requiring liver transplant [1–3, 6–8, 10, 21, 22]. Despite a successful liver transplantation, extra-hepatic manifestations can persist or even worsen. Many patients continue to have intractable watery diarrhea, worsening hearing loss, pancreatitis and may develop liver steatosis in the liver graft that can progress to steatohepatitis and even cirrhosis [9, 11–13, 18, 22]. The etiology of the liver steatosis in PFIC1 patients post-OLT and its effect on the graft are not completely understood although the disruption of enterohepatic circulation of bile acids and their effects on FXR have been suggested as possible mechanisms [11–16, 23].

Investigations on the function and structure of the different mutations of the ATP8B1 gene are undergoing. Gonzales et al. found in their study that several of the missense mutations of the ATP8B1 gene had aberrant folding and decreased expression at the plasma membrane that could be leading to steatosis [17, 18]. They proposed that treatment with pharmacological chaperones such as 4-phenylbutyrate, could represent a new strategy in the management to prevent the steatosis [17, 18, 22]. Gene or mutation specific therapy represents a big opportunity for new studies in the near future.

In this study, all of our PFIC1 patients developed steatosis, with 60% developing steatohepatitis and 40% advancing to fibrosis. This is relevant because steatohepatitis can progress to cirrhosis with significant clinical complications, as did one of our patients who developed ascites and portal hypertension within three years after OLT. Interestingly, the lipid profiles in our patients remained uniformly low post-OLT despite the development of the steatosis. Obesity is a common finding in children post liver transplant that can lead to FLD. Perito et al. published analysis on the United Network for Organ Sharing (UNOS) data and reported that the weight status at transplant was the strongest and most consistent factor associated with obesity post transplant. They also reported that certain risk factors such as ethnicity and race play a role [24, 25]. Although all our PFIC1 group developed steatosis post-OLT, none of them had any of these risk factors. All of them were Amish and had normal BMIs pre and post-transplant.

In order to investigate mechanistic pathways leading to the development of FLD, we used breath testing for volatile organic compounds by selective ion flow tube mass spectrometry (SIFT-MS). The composition of breath is a reflection of the metabolomes in the blood. Breath testing has many clinical uses such as asthma, transplant organ rejection, detection of fatty liver disease, *Helicobacter pylori* infection, detection of lung cancer, among others [19, 26–29]. This is a novel technique that is inexpensive, rapid and most importantly non-invasive, that can provide valuable information about the metabolic condition of an individual [27–29]. In our study, we analyzed four VOCs: acetaldehyde, isoprene, ammonia and pentane. Acetaldehyde, a marker of endogenous alcohol production by the gut microflora was markedly elevated in the PFIC1 group [19, 27, 28]. Isoprene reflects the cholesterol synthesis and this was lower in our PFIC1 patients, maybe this signifies that cholesterol synthesis is lower in PFIC1 patients [19, 27, 28]. Ammonia, which reflects more advance liver disease, was higher in the PFIC1 group; as well as pentane, which is a marker of oxidative stress [19, 27, 28, 30, 31]. These might be associated with the development of FLD in the patients with PFIC1 post-OLT but should be investigated in further studies.

The strengths in our study are that our patients were all PFIC1 diagnosed by liver biopsy. All the biopsies were reviewed by an expert liver pathologist; we used a non-bias and non-invasive technique with the breath testing for VOC. Our study has several limitations that include a small sample, our PFIC1 patients were diagnosed clinically and histopathologically and none had genetic confirmatory testing, breath testing results could have been affected by the diet or environmental exposure of the patients and that the VOC were taken at a single point in time.

CONCLUSION

In conclusion, fatty liver disease (FLD) was a consistent finding in orthotopic liver transplantation (OLT) recipients for PFIC1 disease. These patients were not obese and had low lipid and glucose levels despite the presence of FLD. Mechanistic pathways leading to FLD in patients with PFIC1 post-OLT should be continued to be investigated in future studies to prevent progression to steatohepatitis, cirrhosis and the need for re-transplantation.

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Author Contributions

Ana Catalina Arce-Clachar – Substantial contributions to conception and design, Acquisition of data, Analysis and interpretation of data, Drafting the article, Revising it critically for important intellectual content, Final approval of the version to be published
Jonathan Moses – Substantial contributions to conception and design, Acquisition of data, Analysis and interpretation of data, Drafting the article, Revising it critically for important intellectual content, Final approval of the version to be published
Gursimran Kochlar – Acquisition of data, Revising it critically for important intellectual content, Final approval of the version to be published
Peggy George – Substantial contributions to conception and design, Acquisition of data, Revising it critically for important intellectual content, Final approval of the version to be published
Vera Hupertz – Analysis and interpretation of data, Revising it critically for important intellectual content, Final approval of the version to be published
Kadakkal Radhakrishnan – Analysis and interpretation of data, Revising it critically for important intellectual content, Final approval of the version to be published
Raed Dweik – Substantial contributions to conception and design, Acquisition of data, Revising it critically for important intellectual content, Final approval of the version to be published
Naim Alkhouri – Substantial contributions to conception and design, Acquisition of data, Analysis and interpretation of data, Drafting the article, Revising it critically for important intellectual content, Final approval of the version to be published

Guarantor

The corresponding author is the guarantor of submission.

Conflict of Interest

Authors declare no conflict of interest.

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