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ORIGINAL ARTICLE

PHENOTYPIC AND GENOTYPIC CHARACTERISTICS OF PROGRESSIVE FAMILIAL INTRAHEPATIC CHOLESTASIS TYPE 3 IN PEDIATRIC POPULATION IN PAKISTAN

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ABSTRACT

OBJECTIVES: To determine the phenotypic and genotypic characteristics of progressive familial intrahepatic cholestasis (PFIC) type 3 in Pakistani children in a hospital setting.

METHODS: This cross-sectional observational study was conducted at department of Pediatrics Gastroenterology & Hepatology, The Children's Hospital Lahore, Pakistan from October 2020 to March 2021. Patients of either sex under 16 years of age presenting with jaundice, pruritus, neonatal cholestasis or with chronic liver and gamma glutamyl transferase >100 IU/ml were included in the study after taking informed consent by parents. For Molecular genetics 2ml blood in EDTA was sent to an international laboratory free of cost on research basis. Reports were assessed and levels were noted and genetic coding was also recorded. Data was entered and analyzed in SPSS version 22. Molecular data was interpreted with the help of clinical geneticist.

RESULTS: Out of 34 children, 14 (41.2%) were males and 20 (58.8%) were females. Mean age of children was 6.71±3.10 years. Consanguinity was noted in 32 (94.1%) parents having positive family history in 24 (70.6%) cases. The most common mutation was c. 1783C>T p.(Arg595*), noted in 12 (35.3%) cases, followed by c. 2861G>T p.(Gly954 ASP) [8 (23.5%) cases], c. 153G>A p.(Trp51) [3 (8.8%) cases], c. 1714 C>T p.(Gln572*) c. 1906C>T p. (Gln636), c. 3220G>A p.(Gly1074Arg, c. 3433del p. (val1145Leufsx7) in 2 (5.9%) cases each, c. 3859 C>T p.(1287Argext*) c. 88-91del p.(Lys30gly fsx7) and c. 1429c>T p. (Gln477) in one (2.9%) case each.

CONCLUSION: Children with PFIC type 3 have variable phenotypic and genotypic presentation.

KEYWORDS: Cholestasis, Progressive Familial Intrahepatic 3 (MeSH); Serum γ-glutamyl transferase (Non-MeSH); ABCB4 variant (Non-MeSH).

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INTRODUCTION

The autosomal recessive condition progressive familial intrahepatic cholestasis (PFIC) is one of the most prevalent causes of chronic liver disease, about 10-15% of children requiring liver transplantation.[1] There are five subtypes (PFIC 1-5) that have been identified. According to serum gamma glutamyl transferase (GGT) activity, they are divided into two categories: low GGT cholestasis and high GGT cholestasis.[2] In contrast to the low GGT found in all other forms of PFIC, PFIC3 has a substantially increased serum GGT. [3] Jaundice, mild to severe pruritus, colored stools, hepatomegaly and splenomegaly are among the symptoms that are experienced by the patients.[4,5]

Mutations in both alleles of the ABCB4/MDR3 gene, which codes for the phospholipid transporter MDR3, which is expressed in the canalicular (apical) membrane of hepatocytes, cause PFIC3. Biallelic or monoallelic ABCB4 defects have been linked to a variety of human liver diseases, including low phospholipid-associated cholelithiasis syndrome, intrahepatic cholestasis during pregnancy, drug-induced liver injury, transient neonatal cholestasis, small duct sclerosing cholangitis, and adult biliary fibrosis or cirrhosis.[6] Furthermore, individuals with ABCB4 / MDR3 mutations have been found to develop hepatocellular carcinoma and intrahepatic cholangiocarcinoma.[7]

PFIC3 is a chronic cholestatic condition defined by an abnormally high GGT. Jaundice, pruritus, and hepatosplenomegaly are common symptoms.[8] Pruritus is less severe than other kinds of PFIC. In comparison to other forms of PFIC, PFIC3 often manifests symptoms at a later age (mean of 39 months, range: 1 month to 20.5 years).[9]

Patients with truncated proteins due to homozygous nonsense mutations show symptoms at an average of 8 months of age, whereas patients with homozygous or heterozygous missense mutations show symptoms at a later age, on average at 3.5 years.[10] PFIC3 patients develop progressive peri-portal inflammation and biliary cirrhosis, leading to portal hypertension. PFIC3 has a slower disease development than PFIC1 and PFIC2, with liver failure emerging at a later age.[11] In PFIC3, prolonged cholestasis is linked with substantial copper buildup in liver

tissue and increased urine copper excretion, i.e. observations that coincide with Wilson's disease diagnostic criteria.[12]

PFIC3 is a rare cause of hereditary cholestasis and not well studied in the pediatric population.[13] We aimed to study phenotypic and genotypic characteristics of PFIC3 in Pakistani children in a hospital setting.

METHODS

This cross-sectional observational study was conducted at department of Pediatrics Gastroenterology & Hepatology, The Children's Hospital Lahore, Pakistan from October 2020 to March 2021. It includes both old and new patients. Reporting time of genetic testing is maximum six weeks. Simple random sampling was done every week on filter paper.

Sample size of 34 children was estimated by using 95% confidence level, 11% absolute precision required and anticipated population proportion i.e. 12% in children attending a tertiary care hospital with symptoms.[11] Patients of either gender under 16 years of age

TABLE I: CLINICAL AND LABORATORY FEATURE OF PATIENTS WITH PROGRESSIVE FAMILIAL INTRAHEPATIC CHOLESTASIS TYPE 3

Variables		Frequency (%age) (N=34)
Symptom	Abdominal distension	31 (91.2%)
	Pallor	26 (76.5%)
	Jaundice	25 (73.5%)
	Clubbing	24 (70.6%)
	Ascites	18 (52.9%)
	Pruritic Marks	15 (44.1%)
	Peripheral edema	11 (32.4%)
	Bruises/ petechiae	10 (29.4%)
	Hematemesis	2 (5.9%)
	Spider naevi	2 (5.9%)
	Vitamin D deficiency	11 (32.4%)
Liver consistency	Firm	28 (82.4%)
	Not palpable	6 (17.6%)
Spleen consistency	Firm	29 (85.3%)
	Not palpable	5 (14.7%)
Ultrasound Findings	Hepatomegaly	3 (8.8%)
	Splenomegaly	7 (20.6%)
	Hepatosplenomegaly	22 (64.7%)
	Not done	2 (5.9%)
Liver Biopsy Findings	Cirrhosis with bile duct proliferation	1 (2.9%)
	Congenital hepatic fibrosis	1 (2.9%)
	Focal bile duct proliferation	1 (2.9%)
	Portal fibrosis	4 (11.8%)
	Not done	27 (79.4%)
	Variables	**Mean± Standard deviation**
Physical examination findings	Liver palpable (cm)	3.41±2.09
	Liver total span (cm)	9.76±1.78
	Spleen Palpable (cm)	291±2.01
Laboratory investigations	Total Bilirubin	4.69±4.10
	Direct Bilirubin	3.13±2.85
	ALT (IU/L)	138.32±61.37
	AST (IU/L)	196.12±74.42
	GGT (IU/L)	230.12±67.04
	Alkaline phosphate (IU/L)	625.56±264.15
	Serum Albumin g/dl	3.07±0.75
	PT	19.44±5.98
	INR	1.73±0.57

ALT= Alanine Transaminase; AST= Aspartate Aminotransferase; GGT= Gamma-glutamyl transpeptidase; PT= Prothrombin Time ; INR= International Normalized Ratio.

presenting with jaundice, pruritus, neonatal cholestasis (defined as child with jaundice, dark urine and deranged liver functions tests since neonatal life) or with chronic liver disease(defined as child with shrunken liver, splenomegaly with or without ascites, with or without portal hypertension) and gamma glutamyl transferase >100 IU/ml were included in the study. PFIC3 was defined as when child had neonatal cholestasis or chronic liver disease and high Gamma glutamyl transferase (GGT >100 IU/L). They all underwent genetic confirmation. While children with acute onset liver disease, fulminant hepatic failure or low GGT cholestasis were excluded from the study.

After approval from the institutional review board, all patients meeting inclusion criteria were enrolled in the study. Informed written consent was taken from all the parents or from the guardians. Demographic data including age sex, family history, onset and duration of symptoms were recorded on a proforma. Detailed general physical and systemic examination especially gastrointestinal was performed and recorded. Detailed liver function tests including serum total bilirubin and direct fraction, ALT, AST, ALP, and serum albumin level were analyzed by Cobas c311 automated hepatology analyzer while PT/INR was analyzed by Stago STA coagulation analyzer in our hospital laboratory. For Molecular genetics 2ml blood in EDTA was sent to an international laboratory (Centogene, Germany) free of cost on research basis. Double stranded DNA capture baits against approximately 36.5 Mb of the human coding exome (targeting >98% of the coding RefSeq from the human genome build GRCh37/hg19) was used to enrich target regions from fragmented genomic DNA with the Twist Human Core Exome Plus. Molecular data was interpreted with the help of clinical geneticist.

RESULTS

Out of 34 children, 14 (41.2%) males and 20 (58.8%) were females. The mean age of children was 6.71±3.10 years.

The mean age at onset of symptoms was 3.28±2.38 months, while the mean age at diagnosis of pruritus was 2.56±2.57 years. Consanguinity was noted in 32 (94.1%) parents and family history of cholestasis was noted in 24 (70.6%) cases and family history of chronic liver disease was positive in 24 (70.6%) cases. The mean height of children was 103.96±16.00 cm while mean weight was 17.01±7.36 kg.

On clinical examination, the most common symptom was abdominal distension and it was noted in 31 (91.2%) cases, pallor was found in 26 (76.5%) cases, jaundice in 25 (73.5%) cases, clubbing in 24 (70.6%) cases, ascites in 18 (52.9%) cases, pruritic marks in 15 (44.1%) cases, peripheral edema in 11 (32.4%) cases and bruises / petechiae in 10 (29.4%) cases (Table I).

Vitamin A & E levels are not being done in Pakistan and we looked for the clinical suspicion of Vitamin A deficiency (eg. night blindness, corneal ulcer etc). Vitamin deficiency was suspected in 19 (26.5%) cases. There was no clinical suspicion of Vitamin E in our study.

Liver was palpable about 3.41±2.09 cm while total liver span was 9.76±1.78 cm. liver consistency was firm in 28 (82.4%) cases and spleen consistency was firm in 29 (85.3%) cases. The mean total bilirubin level was 4.69±4.10 IU, total bilirubin level was 3.13±2.85 IU, mean ALT was 138.32±61.37 IU/L while mean AST was 196.12±74.42 IU/L.

On ultrasound, hepatosplenomegaly in 22 (64.7%) cases. On liver biopsy, it was noted that portal fibrosis was most common finding [4 (11.8%) cases]. Congenital hepatic fibrosis was noted and in 1 (2.9%) case, focal bile duct proliferation was noted and in 27 (79.4%) cases biopsy was not done (Table I).

On genetic testing, ABCB4 was detected in all (100%) cases. While the variants of genetic mutations were noted. The most common mutation was c. 1783C>T p. (Arg595*) and it was noted in 12 (35.3%) cases, followed by c. 2861G>T p.(Gly954 ASP) [8 (23.5%) cases], c. 153G>A p.(Trp51) [3 (8.8%) cases] (Table II).

TABLE II: SPECTRUM OF GENETIC VARIANTS IN PATIENTS WITH PROGRESSIVE FAMILIAL INTRAHEPATIC CHOLESTASIS TYPE 3

SPECTRUM OF GENETIC VARIANTS		Frequency (%)
Feature	ABCB4 gene mutation	34 (100%)
Variants	c.1783C>T p.(Arg595*)	12 (35.3%)
	c. 2861G>T p.(Gly954 ASP)	8 (23.5%)
	c.153G>A p.(Trp51)	3 (8.8%)
	c.1714 C>T p.(Gln572*)	2 (5.9%)
	c.1906C>T p. (Gln636)	2 (5.9%)
	c. 3220G>A p.(Gly1074Arg)	2 (5.9%)
	c. 3433del p. (val1145Leufsx7)	2 (5.9%)
	c. 3859 C>T p.(1287Argext*)	1 (2.9%)
	c. 88-91del p.(Lys30gly fsx7)	1 (2.9%)
	c.1429c>T p. (Gln477)	1 (2.9%)

ABCB4= ATP Binding Cassette Subfamily B Member 4.

DISCUSSION

PFIC is an autosomal recessive category of liver disorders that manifest as the neonatal cholestasis.[15] It is a genetic cholestatic liver condition that commonly leads to liver failure in children. Despite the fact that it has the potential to cause considerable morbidity, it has received little research. Preliminary observations made at our facility while dealing with similar patients have led us to believe that PFIC is not as uncommon as stated in Western literature. The problem is exacerbated by a lack of awareness of the true illness burden in Asian nations and a lack of data on genotype-phenotype association.[15,16]

Although the actual prevalence is unclear, the incidence is believed to be between 1 in 50,000 and 1 in 100,000 births.[17] In three investigations, the prevalence of PFIC in the local community was found to range from 9.0 to 12.0% of children hospitalized with

cholestasis, acute liver failure, or splenomegaly.[14] Early diagnosis and therapy can be aided by proper clinicopathologic correlation and genetic testing.[15]Pediatricians have a clinical challenge when it comes to differentiating newborn cholestasis.[18]

PFIC is a group of uncommon hereditary diseases caused by faulty bile secretion pathways. The illness is generally identified in the early years of childhood and commonly presents with signs and symptoms of intrahepatic cholestasis such as pruritus, dark urine, pale stool, lack of appetite, and weariness. It is usually classified into five subtypes: PFIC type 1 to type 5.[19,20]

In our study, on genetic testing, ABCB4 gene mutation was detected in all (100%) cases of PFIC type 3.While the variants of genetic mutations were noted as shown in table 2.

Intraoperative cholangiography revealed biliary atresia in one trial. Genetic testing revealed the ABCB4 gene mutation IVS13+6G>A/G. The patient was diagnosed with PFIC3 and biliary atresia.[18]In individuals with biliary atresia who experienced persistent jaundice following Kasai portoenterostomy, further tests, such as genetic testing, should be undertaken to rule out intrahepatic cholestasis.[18]

Through genetic research, cholestasis has been deconstructed. The main PFIC genes have now been identified. PFIC1 is encoded by ATP8B1, BSEP is encoded by ABCB11, MDR3 is encoded by ABCB4, TJP2 is encoded by TJP2, FXR is encoded by NR1H4, and MYO5B is encoded by MYO5B. The complete range of symptoms associated with mutations in each gene, as well as our knowledge of disease processes, are presented. Therapeutic response differences are becoming apparent, as are treatment objectives for the future.[21]

The mean GGT level in our research was 230.12±67.04 IU/L. In patients with PFIC1/2, serum GGT is normal, but in those with PFIC3, it is elevated. PFIC patients suffer from severe symptoms and have a dismal prognosis.[23] To further improve patient management and clinical trial design, more research is needed. There is a scarcity of evidence on the epidemiology and socioeconomic impact of PFIC3. To make gene therapy for all kinds of PFIC a reality, substantial barriers must be overcome using currently available gene therapy vectors.[24]

CONCLUSION

It has been concluded that the frequency of genetic mutations is high in children with PFIC and ABCB4 was noted in all 100% cases and the most common genetic mutation was c. 1783C>T p.(Arg595*). Thus, we have observed a distinct type of Phenotypic and Genotypic characteristics of children diagnosed with PFIC3. Though we have different genetic mutations but general presentation was same.

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AUTHOR'S CONTRIBUTION

Following authors have made substantial contributions to the manuscript as under:

MA: Acquisition of data, drafting the manuscript, critical review, approval of the final version to be published

HAC & IM: Conception and study design, critical review, approval of the final version to be published

MAA & NW: Acquisition of data, drafting the manuscript, critical review, approval of the final version to be published

MNA: Analysis and interpretation of data, critical review, approval of the final version to be published

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

CONFLICT OF INTEREST

Authors declared no conflict of interest

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DATA SHARING STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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