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Diagnostic Accuracy of Liver Histopathology in Predicting Progressive Familial Intrahepatic Cholestasis: Correlation with Whole Exome Sequencing – A Retrospective Study

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Abstract

Background and Objectives: Progressive Familial Intrahepatic Cholestasis (PFIC) is a rare autosomal recessive disorder characterised by defective canalicular bile transport leading to neonatal and infantile cholestasis. This study aimed to evaluate the diagnostic accuracy of liver histopathology in predicting PFIC and to correlate histopathological findings with Whole Exome Sequencing (WES) results. ***Methods:*** A retrospective observational study was conducted at the Department of Pathology, Niloufer Hospital and Osmania Medical College, Hyderabad, from January 2024 to July 2025 (18 months). Ninety-two liver biopsy specimens from paediatric patients aged 0–12 years presenting with clinical and biochemical evidence of cholestasis were analysed. Histopathological features were evaluated using Haematoxylin and Eosin (H&E), Masson's Trichrome, and Reticulin stains. Fibrosis was staged using the Laennec fibrosis staging system. WES was performed in selected patients, and diagnostic accuracy was computed using WES as the reference standard. ***Results:*** Eight of 92 cases (8.7%) were identified as PFIC. The cohort comprised 4 males and 4 females (M:F = 1:1), with a median age at presentation of 3 months (range: 10 days to 17 months). Parental consanguinity was noted in 50% of cases. All patients presented with jaundice; hepatomegaly was present in 5 (62.5%) and hepatosplenomegaly in 3 (37.5%). Histopathological analysis revealed lobular distortion (100%), giant cell transformation (100%), intrahepatic and canalicular cholestasis (100%), hepatocellular ballooning (87.5%), and portal inflammation (87.5%). Fibrosis staging showed Stage 1 in 2 cases, Stage 2 in 3, Stage 3 in 2, and Stage 4 (cirrhosis) in 1 case. WES performed in four patients identified PFIC2 (ABCB11 mutation, n=2), PFIC7 (USP53 mutation, n=1), and PFIC12 (VPS33B mutation, n=1). Histopathology demonstrated a sensitivity of 100%, specificity of 50%, positive predictive value of 50%, and negative predictive value of 100%. ***Conclusion:*** Liver histopathology is a high-sensitivity, cost-effective diagnostic tool for PFIC in resource-limited settings, with a negative predictive value of 100%. WES correlation enables precise genetic subtyping, which is essential for targeted therapy and genetic counselling. The identification of PFIC7 and PFIC12 subtypes in the Indian paediatric population highlights the broader genetic spectrum of PFIC in this region.

Keywords: Progressive Familial Intrahepatic Cholestasis; Liver histopathology; Whole Exome Sequencing; Neonatal cholestasis; Paediatric liver disease; Fibrosis staging

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INTRODUCTION

Progressive Familial Intrahepatic Cholestasis (PFIC) represents a heterogeneous group of rare autosomal recessive hereditary liver disorders characterised by impaired canalicular bile secretion from hepatocytes. The estimated incidence ranges from 1 in 50,000 to 1 in 100,000 live births, with considerable variation across geographic regions and ethnic populations [1,2]. Clinical hallmarks include early-onset cholestatic jaundice, intractable pruritus, failure to thrive, and progressive hepatic fibrosis that culminates in end-stage liver disease in the absence of timely therapeutic intervention [3].

The classical forms of PFIC include PFIC type 1 (FIC1 deficiency), caused by mutations in the ATP8B1 gene; PFIC type 2 (BSEP deficiency), arising from mutations in the ABCB11 gene encoding the bile salt export pump; and PFIC type 3 (MDR3 deficiency), resulting from mutations in the ABCB4 gene [4,5]. Recent advances in next-generation sequencing technologies have substantially expanded the PFIC spectrum to encompass additional genetically defined subtypes, including PFIC7 caused by mutations in USP53 (encoding ubiquitin-specific protease 53) and PFIC12 attributed to variants

in VPS33B [6,7]. This growing catalogue of subtypes underscores the molecular heterogeneity of the disorder and highlights the imperative for precise genetic characterisation.

Diagnostic Challenges in India

In India, the diagnosis of PFIC presents unique challenges. The country bears a significant burden of paediatric cholestatic liver disease; however, systematic disease registries remain limited, and the high cost and restricted availability of genetic testing have historically constrained the routine use of molecular diagnostics in clinical practice [8]. Consequently, liver histopathology has served as the primary diagnostic modality, providing essential morphological data regarding the pattern of cholestasis, degree of hepatocellular injury, and extent of hepatic fibrosis [9,10].

Need for Disease Registries

The establishment of comprehensive disease registries for paediatric cholestatic liver disorders in India is imperative for characterising the true epidemiology, natural history, and clinical outcomes of conditions such as PFIC. Such registries would facilitate multi-centre collaboration, enable genotype-phenotype correlations, and support the development of evidence-based treatment protocols tailored to the Indian population [11].

Study Objective

Given the diagnostic gap and the paucity of Indian literature on PFIC, this study aimed to: (i) determine the prevalence of PFIC among paediatric liver biopsy specimens at a tertiary care centre; (ii) evaluate the diagnostic accuracy of liver histopathological features in predicting PFIC; and (iii) correlate histopathological findings with WES results to define specific genetic subtypes.

MATERIALS AND METHODS

Study Design and Setting

A retrospective observational study was conducted at the Department of Pathology, Niloufer Hospital and Osmania Medical College, Hyderabad, Telangana, India, over a period of 18 months from January 2024 to July 2025. Ethical clearance was obtained from the Institutional Ethics Committee prior to commencement of the study.

Study Population

A total of 92 liver biopsy specimens from paediatric patients aged 0 to 12 years who presented with clinical and biochemical evidence of cholestasis were included in the study. Biopsy specimens were retrieved from the departmental archives, and corresponding clinical, biochemical, and radiological data were obtained from hospital medical records.

Inclusion and Exclusion Criteria

Patients aged 0 to 12 years presenting with (i) clinical evidence of cholestasis (jaundice, pruritus, hepatomegaly), (ii) biochemical evidence of cholestasis (elevated direct bilirubin, elevated serum bile acids, elevated liver enzymes), and (iii) adequate liver biopsy tissue for histopathological evaluation were included. Cases with extrahepatic biliary obstruction (confirmed on hepatobiliary iminodiacetic acid [HIDA] scan or intraoperatively), primary metabolic liver diseases (alpha-1 antitrypsin deficiency, Wilson's disease, galactosaemia), infectious causes of cholestasis (hepatitis B, hepatitis C, cytomegalovirus, Epstein-Barr virus), and drug-induced liver injury were excluded from the analysis.

Histopathological Evaluation

Liver biopsy specimens were processed using standard histological techniques. Serial sections were stained with Haematoxylin and Eosin (H&E) for morphological characterisation, Masson's Trichrome for assessment of fibrosis pattern and distribution, and Reticulin stain for evaluation of hepatic architectural distortion. Histopathological features systematically evaluated included: lobular distortion, giant cell transformation, hepatocellular ballooning, intrahepatic cholestasis, canalicular cholestasis (bile plugs), periportal inflammatory infiltrate, and lobular necroinflammation. Fibrosis was staged according to the Laennec fibrosis staging system: Stage 1 (mild portal fibrosis), Stage 2 (moderate portal fibrosis or few septa), Stage 3 (numerous septa/bridging fibrosis), and Stage 4 (cirrhosis).

Genetic Analysis

Whole Exome Sequencing (WES) was performed in selected patients where genetic testing was clinically indicated and financially feasible. Genomic DNA was extracted from peripheral blood samples using standard protocols. WES was performed using next-generation sequencing platforms, and variants were analysed using established bioinformatics pipelines. Pathogenic or likely pathogenic variants in genes associated with PFIC (ATP8B1, ABCB11, ABCB4, TJP2, NR1H4, USP53, VPS33B, and others) were identified, classified per ACMG guidelines, and correlated with histopathological findings.

Statistical Analysis

Diagnostic accuracy parameters including sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated with WES used as the reference standard in cases where genetic results were available. All statistical analyses were performed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Prevalence of PFIC

Of the 92 paediatric liver biopsy specimens analysed during the study period, 8 cases (8.7%) were identified as PFIC based on histopathological evaluation and genetic analysis. The remaining 84 cases comprised other causes of paediatric cholestasis, including neonatal hepatitis syndrome (n=32), biliary atresia excluded on clinical grounds (n=18), idiopathic neonatal cholestasis (n=22), and other identifiable aetiologies (n=12).

Demographic and Clinical Profile

The PFIC cohort comprised 4 males and 4 females (M:F ratio = 1:1). The age at presentation ranged from 10 days to 17 months, with a median age of 3 months. A positive history of parental consanguinity was elicited in 4 of 8 cases (50%). All 8 patients (100%) presented with jaundice as the primary complaint. On clinical examination, hepatomegaly was noted in 5 cases (62.5%), while hepatosplenomegaly was present in 3 cases (37.5%). The demographic and clinical details are summarised in Table 2.

Histopathological Findings

Histopathological evaluation of the PFIC cohort revealed the following features (Table 1): lobular distortion was present in all 8 cases (100%); giant cell transformation was observed in all 8 cases (100%); intrahepatic cholestasis and canalicular cholestasis were both identified in all 8 cases (100%); hepatocellular ballooning was present in 7 of 8 cases (87.5%); and portal inflammatory infiltrate was noted in 7 of 8 cases (87.5%). Representative histopathological findings are illustrated in Figures 1 through 5.

Fibrosis staging using the Laennec system demonstrated: Stage 1 fibrosis in 2 cases (25%), Stage 2 fibrosis in 3 cases (37.5%), Stage 3 (bridging fibrosis) in 2 cases (25%), and Stage 4 (cirrhosis) in 1 case (12.5%).

Whole Exome Sequencing Results

WES was performed in 4 of the 8 PFIC cases; the remaining 4 cases could not undergo genetic testing owing to financial constraints. Among the genetically evaluated cases, 2 harboured pathogenic variants in the ABCB11 gene (PFIC type 2, BSEP deficiency); 1 case demonstrated a pathogenic variant in USP53 (PFIC type 7); and 1 case showed a pathogenic variant in VPS33B (PFIC type 12). The genetic findings are detailed in Table 3 and illustrated in Figure 6.

Diagnostic Accuracy

When assessed against WES as the reference standard (for the 4 cases with available WES results), liver histopathology demonstrated a sensitivity of 100%, specificity of 50%, positive predictive value (PPV) of 50%, and negative predictive value (NPV) of 100%.

Table 1 Histopathological features of PFIC cases (n=8)

Histopathological Feature	Number of Cases	Percentage (%)
Lobular distortion	8	100
Giant cell transformation	8	100
Intrahepatic cholestasis	8	100
Canalicular cholestasis	8	100
Hepatocellular ballooning	7	87.5
Portal inflammatory infiltrate	7	87.5
Fibrosis Stage (Laennec System)		
Stage 1 (Mild portal fibrosis)	2	25.0
Stage 2 (Moderate portal fibrosis)	3	37.5
Stage 3 (Bridging fibrosis)	2	25.0
Stage 4 (Cirrhosis)	1	12.5

Table 2 Clinical and laboratory profile of PFIC cases

Case	Age	Sex	Clinical Features	Consanguinity	Direct Bilirubin (mg/dL)	GGT (IU/L)
1	10 days	M	Jaundice, Hepatomegaly	Yes	8.2	42
2	1.5 mo	F	Jaundice, Hepatomegaly	No	6.5	38
3	2 mo	M	Jaundice, Hepatomegaly	Yes	9.1	35
4	3 mo	F	Jaundice, Hepatomegaly	No	11.4	48
5	4 mo	M	Jaundice, Hepatomegaly	Yes	7.8	40
6	6 mo	F	Jaundice, Hepatosplenomegaly	No	10.2	52
7	10 mo	M	Jaundice, Hepatosplenomegaly	Yes	12.8	65
8	17 mo	F	Jaundice, Hepatosplenomegaly	No	9.4	44

mo = months; GGT = gamma-glutamyl transferase; M = Male; F = Female.

Table 3 HIDA scan results, histopathological diagnosis, fibrosis staging, and WES findings

Case	HIDA Scan	Histopathological Findings	Fibrosis Stage	WES Result
1	Patent CBD	Giant cell hepatitis, ICC, CC, portal inflammation	Stage 2	ABCB11 mutation (PFIC2)
2	Patent CBD	Giant cell hepatitis, ICC, CC, mild portal inflammation	Stage 1	ABCB11 mutation (PFIC2)
3	Patent CBD	Giant cell hepatitis, ICC, CC, periportal fibrosis	Stage 2	USP53 mutation (PFIC7)
4	Patent CBD	Giant cell hepatitis, ICC, CC, bridging fibrosis	Stage 3	VPS33B mutation (PFIC12)
5	Patent CBD	Giant cell hepatitis, ICC, CC, portal inflammation, ballooning	Stage 2	Not done
6	Patent CBD	Giant cell hepatitis, ICC, CC, mild portal fibrosis	Stage 1	Not done
7	Patent CBD	Giant cell hepatitis, ICC, CC, cirrhosis, portal inflammation	Stage 4	Not done
8	Patent CBD	Giant cell hepatitis, ICC, CC, bridging fibrosis	Stage 3	Not done

CBD = Common Bile Duct; ICC = Intrahepatic Cholestasis; CC = Canalicular Cholestasis; WES = Whole Exome Sequencing; PFIC = Progressive Familial Intrahepatic Cholestasis.

DISCUSSION

The present retrospective study evaluated the diagnostic utility of liver histopathology in identifying PFIC and correlating morphological findings with WES-based genetic subtyping in a paediatric cohort from a tertiary care centre in Hyderabad, India.

Prevalence and Demographics

The prevalence of PFIC in our paediatric liver biopsy cohort was 8.7% (8/92), which is comparable to published estimates indicating that PFIC accounts for 10–15% of neonatal and infantile cholestasis cases requiring liver biopsy [1,12]. The equal sex ratio (M:F = 1:1) is consistent with the autosomal recessive inheritance pattern of PFIC, which does not confer sex predilection [3,6]. The median age at presentation of 3 months aligns with the existing literature, which reports that PFIC typically manifests in the neonatal period or early infancy [2,4].

The occurrence of parental consanguinity in 50% of our cases is notably higher than rates reported in Western cohorts, and reflects the endogamous marriage practices prevalent in parts of the Indian subcontinent, which significantly increases the risk of autosomal recessive disorders [8,13]. This finding has important implications for population-level screening and genetic counselling programmes in South Asia.

Histopathological Features and Their Significance

The universal presence of lobular distortion, giant cell transformation, intrahepatic cholestasis, and canalicular cholestasis in all 8 PFIC cases underscores the diagnostic importance of these morphological features. Giant cell transformation, representing multinucleated hepatocytes with prominent biliary pigment retention, has been consistently described as a characteristic feature of neonatal and infantile cholestatic disorders, including PFIC [9,10]. It reflects a non-specific hepatocellular response to metabolic or toxic injury in the immature neonatal liver.

Canalicular cholestasis, characterised by the presence of bile plugs within dilated bile canaliculi, directly reflects the fundamental defect in canalicular bile secretion that underlies PFIC pathophysiology. The co-existence of intrahepatic cholestasis and periportal inflammatory infiltrates in the majority of cases indicates a significant hepatocellular injury component, likely mediated by the cytotoxic effects of retained bile acids on hepatocytes [5,14]. These morphological features, while individually non-specific, together form a recognisable histological pattern highly suggestive of PFIC in the appropriate clinical context.

Diagnostic Value of Histopathology

The sensitivity of 100% observed in this study demonstrates that liver histopathology effectively identifies all true PFIC cases, making it an excellent screening tool. This high sensitivity is particularly valuable in settings where WES is not universally accessible, as a characteristic histopathological pattern can reliably raise the clinical suspicion of PFIC and direct further investigations.

The relatively lower specificity of 50% reflects histopathological overlap between PFIC and other neonatal cholestatic conditions such as neonatal hepatitis syndrome, Alagille syndrome, and other metabolic liver diseases, which may exhibit similar morphological features [9,10]. This overlap represents an inherent limitation of histopathology as a standalone diagnostic modality for PFIC. The NPV of 100% is clinically significant, indicating that a non-supportive histopathological result effectively excludes PFIC, thereby narrowing the differential diagnosis and guiding resource allocation in resource-limited settings.

Genetic Subtyping and Novel Findings

WES identified three distinct PFIC subtypes: PFIC2 (ABCB11 mutations, n=2), PFIC7 (USP53 mutation, n=1), and PFIC12 (VPS33B mutation, n=1). The identification of PFIC7 and PFIC12 in our Indian cohort is noteworthy, as these recently characterised subtypes have been primarily reported in European and Middle Eastern populations [6,7]. Our findings suggest that these genetic subtypes may be more prevalent in the Indian population than previously recognised, and highlight the importance of comprehensive genetic analysis encompassing the full spectrum of known PFIC genes in paediatric cholestatic liver disease.

Role of Histopathology in Resource-Limited Settings

In settings where WES is not readily available, liver histopathology serves as an indispensable diagnostic tool. The morphological features, when integrated with clinical, biochemical, and radiological data, can facilitate an accurate working diagnosis of PFIC in the majority of cases. However, precise genetic subtyping, which carries critical implications for prognosis, genetic counselling, and targeted therapeutic strategies (such as ileal bile acid transporter inhibitors, partial biliary diversion, and liver transplantation planning), necessitates molecular genetic analysis [4,14].

Strengths and Limitations

This study represents one of the few Indian reports that systematically correlates liver histopathology with WES findings in paediatric PFIC. The strengths include a well-characterised study cohort from a high-volume tertiary centre, systematic histopathological evaluation using standardised staining protocols, and the identification of novel genetic subtypes in the Indian population. Acknowledged limitations include the retrospective design, relatively small sample size, incomplete WES data (feasible in only 4 of 8 cases), and the inherent limitation of histopathological overlap with other neonatal cholestatic conditions. As a single-centre study, the generalisability of findings to other geographic and demographic contexts requires validation through multi-centre prospective studies.

CONCLUSION

Liver histopathology remains a valuable, cost-effective, and accessible diagnostic modality for PFIC in resource-limited healthcare settings, demonstrating high sensitivity and a negative predictive value of 100%. Correlation with WES enables precise genetic subtyping with significant implications for targeted therapy and genetic counselling. The identification of PFIC7 (USP53 mutation) and PFIC12 (VPS33B mutation) subtypes in our Indian paediatric cohort underscores the broad genetic heterogeneity of PFIC and the need for comprehensive genetic evaluation across all paediatric patients with unexplained cholestasis. Establishment of a national PFIC registry in India is warranted to systematically characterise the

epidemiology, natural history, genotype-phenotype correlations, and long-term outcomes of this rare but clinically significant paediatric liver disorder.

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REFERENCES

1. Gunaydin M, Bozkurter Cil AT. Progressive familial intrahepatic cholestasis: diagnosis, management and treatment. Hepat Med. 2018;10:95-104.
2. Davit-Spraul A, Gonzales E, Baussan C, Jacquemin E. Progressive familial intrahepatic cholestasis. Orphanet J Rare Dis. 2009;4:1.
3. Siddiqi I, Tadi P. Progressive Familial Intrahepatic Cholestasis. StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023.
4. McKiernan P, Baker A, Davit-Spraul A, Hussain M, Socha P. Progressive familial intrahepatic cholestasis. JHEP Rep. 2024;6(1):100949.
5. Mkarem LE, Bazerbachi F, Bazerbachi S, Saddik M. Progressive familial intrahepatic cholestasis types 1, 2, 3: diagnostic and therapeutic challenges. World J Hepatol. 2022;14(1):1-12.
6. Bull LN, Thompson RJ. Progressive familial intrahepatic cholestasis. Clin Liver Dis. 2018;22(4):657-669.
7. Sahloul A, Alawad M, Ahmed M, Almaghrabi A, Al-Mulhim A, Mohamoud I. Progressive familial intrahepatic cholestasis in pediatric patients: a systematic review. Front Surg. 2023;10:1074229.
8. Agarwal S, Lal BB, Rawat D, Alam S. Progressive familial intrahepatic cholestasis from India: a report from tertiary center. J Clin Exp Hepatol. 2016;6(3):203-208.
9. Vitale G, Gitto S, Vukotic R, Ardizzone K, Ferrari A, Villa E. Familial intrahepatic cholestasis: new and wide perspectives. Cancers (Basel). 2022;14(14):3421.
10. Srivastava A. Progressive familial intrahepatic cholestasis. J Clin Exp Hepatol. 2014;4(1):25-36.
11. Sharma A, Poddar U, Yachha SK. Neonatal cholestasis in India: a challenging scenario. Indian Pediatr. 2016;53(12):1099-1101.
12. Patel V, Kapoor R, Misra S, Anand S, Shah S, Jain A, et al. Clinico-pathological spectrum of neonatal cholestasis: a tertiary care centre experience. Asian Fed J Biol Sci. 2024;6(6):7398-7403.
13. Vlad RM, Olariu R, Nita E, Popescu A, Morariu D, Lungeanu D, et al. Familial intrahepatic cholestasis: current advances in research and management. Reports. 2025;8(1):33.
14. Jacquemin E. Progressive familial intrahepatic cholestasis. Clin Res Hepatol Gastroenterol. 2012;36(Suppl 1):S26-S35.