Original Article

Neonatal sclerosing cholangitis

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ABSTRACT

Background: Neonatal sclerosing cholangitis (NSC) is a rare autosomal recessive disorder with severe liver disease caused by homozygous or compound heterozygous mutation in DCDC2/CLDN1 mutations. We aim to describe the clinical findings and molecular data in eight Pakistani children with DCDC2 and CLDN1 mutations.

Methods: This is a retrospective observational study conducted at the department of Pediatric Gastroenterology & Hepatology, The Children's hospital & University of Child Health Sciences, Lahore, Pakistan. All cases of NSC confirmed on molecular testing, under 18-year of age were included in the study. Their demographic, clinical and laboratory data were collected and analyzed.

Results: There were total ten children from seven unrelated families with the genetic diagnosis of neonatal sclerosing cholangitis. Eight had biallelic mutations in DCDC2 and two had CLDN1 mutations confirming the diagnosis of NSC. There were eight males with 100% consanguinity rate. The mean age at diagnosis was 7.3±3.4 years, while the mean age of onset of symptoms was 1.8±0.75 months. All of them had neonatal cholestasis and progressively increasing jaundice after neonatal age with acholic stools and progressed to chronic liver disease without any clear diagnosis. Their mean liver and spleen span were 7.6±1.0 cm and 6.5±2.5 cm respectively. Hyperbilirubinemia with markedly elevated GGT, deranged liver enzymes and histopathological diagnosis of portal fibrosis were the main laboratory findings. Endoscopic findings of early portal hypertension were noted. All were managed with fat soluble vitamins, ursodeoxycholic acid and successive endoscopic band ligation and were stable except two children requiring liver transplant in the last two years.

Conclusion: Neonatal sclerosing cholangitis is a rare condition presenting with jaundice, pale stools, severe liver disease and elevated GGT in highly consanguineous families. Molecular testing can make a difference among other conditions presenting with similar symptoms.

Keywords: Neonatal sclerosing cholangitis, Children, Mutation

INTRODUCTION

Neonatal Sclerosing Cholangitis (NSC) is a rare autosomal recessive disorder characterized by severe liver disease with onset in infancy. NSC has progressive course like biliary atresia with worsening of jaundice from infancy, acholic stools, progressive liver dysfunction leading to early fibrosis and cirrhosis of liver but unlike biliary atresia it has patent bile ducts on cholangiogram with bile duct irregularities and only few

require liver transplant in first few decades [1]. Few syndromes are associated including Kabuki syndrome and neonatal ichthyosis-sclerosing cholangitis syndrome [2]. NSC and biliary atresia present similarly in the neonatal age with cholestasis, pale stools and high GGT levels and even sometimes difficult to differentiate radiologically and on histology between these two conditions [3,4].

The diagnostic workup includes transaminesemia, high gamma glutamyl transferase (GGT), and cholangiogram showing thin irregular bile ducts [4,5]. Liver biopsies findings are ductular proliferation, portal tract inflammation, and bridging fibrosis, and most patients developed biliary cirrhosis. Other histologic findings included ductal bile plugs, variable ectasia of perihilar bile ducts, absence of bile ducts in areas of fibrosis, and chronic cholestasis [5].

Most of the patients develop early portal hypertension and cirrhosis requiring transplant in second decade. In the present study, we report the clinical findings, laboratory data, and molecular genetic analysis in ten patients with DCDC2/ CLDN1 mutations. This is the first report of DCDC2/ CLDN1 mutations from Pakistan.

METHODS

This is a retrospective, observational study conducted at the department of Pediatric Gastroenterology & Hepatology, The Children's hospital & University of Child Health Sciences, Lahore, Pakistan. We aim to describe the clinical findings and molecular data in ten Pakistani children with DCDC2 and CLDN1 mutations. All cases of neonatal sclerosing cholangitis confirmed on molecular testing, under 18-year of age were included in the study. This study was approved by Institutional Review Board, conducted in accordance with the Declaration of Helsinki. Only those children having genetic analysis done and confirmed as neonatal sclerosing cholangitis with any of the following,

1. Neonatal cholestasis which is defined as persistent conjugated hyperbilirubinemia for more than two weeks, acholic stools and high GGT.

2. Cholangiogram: Patent biliary tract with thin irregular bile ducts.

3. Liver biopsy: Portal fibrosis and cirrhosis.

We excluded all those children with biliary atresia, metabolic and genetic liver diseases, infectious causes and choledochal cyst.

Ethical Approval: Approval was obtained from the Institutional Review Board (IRB) dated 16-06-2021 ref no 2021-294-CHICH.

Data including age at diagnosis, age at first symptom, family history, physical examination (anthropometry, liver and spleen span), laboratory parameters including serum transaminases, ultrasonography, histologic and endoscopic findings) were reviewed. The complications of the disease including development of chronic liver disease by Child Pugh Score system were documented. Statistical analysis was carried out by using the Statistical Package for Social Sciences version 23 (SPSS Chicago, IL, USA). Simple descriptive statistics were used. Mean and standard deviations (SD) were calculated for quantitative variables like age and duration of illness. Frequencies and percentages were calculated for qualitative variables like gender and clinical characteristics.

Molecular study

2.5 ml of peripheral blood was withdrawn from patients and parents in standard ethylene diamine tetra acetic acid (EDTA) tube and sent to Centogene, Germany. Where genomic DNA was enzymatically fragmented and entire coding region of DCDC2 (NM_016356.5) /CLDN1 (NM_021101.4) plus approximately 10 base pairs of flanking non-coding intronic DNA on either side of each exon was targeted. All single nucleotide variants (SNVs) were reported by following the recommendation of Human Genome Varia1tion Society (HGVS) (http://www.hgvs.org/mutnomen). The identified mutations were annotated against publicly available database such as the Human Genome Mutation Database (HGMD) (http://www.hgmd.cf.ac.uk/ac/index.php) and 1000 Genome Browser (http://www.1000genomes.org).

The pathogenicity and functional effects of the new DCDC2/CLDN1mutations were evaluated by using different software. The detected mutations were also compared with HomoloGene (NCBI) database (https://www.ncbi.nlm.nih.gov) to evaluate the percentage of conservation among eukaryote species.

RESULTS

There were 10 (10.7%) patients from seven unrelated families diagnosed as NSC out of 93 genetic cholestasis disorders and cryptogenic liver diseases. There were 8(80%) males with very high consanguinity rate approaching to 100%. The mean age at diagnosis was 7.3±3.4 years, while the mean age of onset of symptoms was 1.8+0.75 months. DCDC2 mutation was found in eight and CLDN1 mutation in two patients. All these children had progressively increasing jaundice with firm hepatosplenomegaly of variable measurements (7.6±1.0 cm and 6.5±2.5 cm) and endoscopic findings of early portal hypertension. Deranged liver functions with markedly elevated GGT and cholestasis were the main biochemical findings. A summary of biochemical, radiological and histopathological findings is given in Table 1.

Family 1: The first and second patients (P1 & P2) were two siblings and related to consanguineous parents. The female P1 and male P2 were 18 years and 8 years of age respectively. Both of them was referred as chronic liver disease due to splenomegaly, mild icterus, elevated transaminases, GGT and portal hypertension. The P2 had neonatal cholestasis and developed significant portal hypertension confirmed on doppler studies and endoscopy. They were labeled as cryptogenic liver disease and now on molecular testing diagnosed neonatal sclerosing cholangitis.

Family 2: The 3rd and 4th patients (P3 & P4) were siblings of consanguineous parents. P3 was 9-year-old boy and P4 was 7-year-old boy when referred to our department. Both patients had neonatal cholestasis, acholic stools extensively worked up for biliary atresia and other causes of neonatal cholestasis. They both had significant portal hypertension required variceal band ligation because of bleeding esophageal varices. P3 & 4 both developed complication of hepatopulmonary syndrome required liver transplantation and is doing very well after transplant.

Family 3: Fifth patient (P5) and sixth patient (P6) were diagnosed at the age of 2 and 3 years respectively. They had persistent conjugated hyperbilirubinemia, acholic stools, itching and abdominal distention started in early infancy. The laboratory parameters showed elevated liver enzymes and GGT. P5 liver biopsy was performed at 2-month of age showed bile duct proliferation and fibrosis.

Family 4 & 5: Seventh patient (P7) and eighths patient (P8) had symptoms of cholestasis with hepatosplenomegaly since early infancy and were referred as CLD with portal hypertension. GGT was high with deranged liver functions and diagnosed at the age of 2 and 2.5 years respectively as NSC on molecular testing.

Family 6: Patient 9 (P9) was born premature, product of consanguineous parents, suspected as a case of chanarin dorfman syndrome on the basis of jaundice, acholic stools, icthyotic skin, sparse thin hair, alopecia and severe pruritis. Laboratory parameters showed hyperbilirubinemia, elevated liver enzymes, elevated GGT, and liver biopsy revealing periportal fibrosis and ductular proliferation.

Family 7: Patient 10 (P10) is 3-year-old and had neonatal cholestasis with pale stools and dark colored urine. His symptoms are persisting and now developed hepatosplenomegaly with evidence of portal hypertension. He has high GGT cholestasis with preserved synthetic functions. Liver biopsy showed features of intrahepatic cholestasis with ductular proliferation. Molecular testing showed CLDN1 mutation favouring Kabuki syndrome associated with NSC.

Molecular analysis

Genetic analysis revealed five novel mutations in DCDC2 in five families (F1-F5); four were missense variables and one deletion. A duplication in exon 3 of CLDN1 (c.461dup) resulting in frameshift and downstream stop codon (p.Val155Serfs*186) were reported in two families (F4 & F5); parents of both families were asymptomatic carriers. In patient 1-4, a homozygous c.203C>G (p.Pro68Arg) novel transversion was detected in DCDC2 in exon 1. Proline at this position is highly conserved across species. The mutation is located within a domain, annotated in UniProt as Doublecortin 1. In patient 7 & 8 a deletion in chromosome 6 encompassing exon 9 in DCDC2 was reported. Six gross mutations have already been reported in DCDC2 in The Human Gene Mutation Database. In patient 9&10 novel transition c.559C>T (p.Leu187Phe) was reported in exon 5 of DCDC2. Leucine at this position is highly conserved across species. The mu

tation is located within a domain, annotated in UniProt as Doublecortin 2. In patient 9 &10 c.461dup; p.Val155Serfs*186 in CLDN1 was reported.

Table 1: Demographic, biochemical, radiological and histological findings of children with neonatal sclerosing cholangitis.

Clinical parameter	**Mean ±SD**
Neonatal sclerosing cholangitis cases	10/93 (11.7%)
Male n(%)	8 (80)
Consanguinity n(%)	10 (100)
Age at first symptoms (months)	2.0±0.81
Age at diagnosis (years)	7.3±3.4
Bilirubin (mg/dl)	2.2±1.5
ALT (IU/L)	183±73
AST (IU/L)	144±49
GGT (IU/L)	148±21
Alkaline phosphatase IU/L)	475±159
Albumin (g/dl)	3.35±0.94
Cholangiogram (#3)	Normal
Histological features	Porto-septal fibrosis, bile ductular proliferation and intrahepatic cholestasis

ALT- Alanine amino transferase, AST- Aspartate amino transferase, GGT- Gamma glutamyl transpeptidase.

All these variants are considered to be pathogenic based on the American College of Medical Genetics and Genomics and the association of Molecular Pathology Criteria. Variants are predicted to be pathogenic by using different annotation software; PolyPhen-2, SIFT Align-GVG, BayesDel, FATHMM, DEOGEN2, M-CAP, Mutation taster and Mutation Assessor. Genome AD shows extremely low frequency in data base. Parents of all patients are heterozygous for the respected variants.

DISCUSSION

Neonatal Sclerosing Cholangitis is a rare autosomal recessive disorder, with phenotypic features similar to biliary atresia. Exact prevalence, gender and ethnic distribution are not known in Pakistan due to scarcity of literature about his entity. To the best of our knowledge, this is the first report from Pakistan about NSC among children of genetic cholestasis or cryptogenic liver disease. There have been case reports and series mentioning male gender predominance and geographically Arabs, European and South Asians ethnicity are affected more [6]. Gender predilection is not documented in the literature but in our study 80% were male. It appears to be common among children of consanguineous families, as seen in the current study having 100% consanguinity.

The presentation of NSC is similar to other neonatal cholestasis disorders with jaundice and pale stools and difficult to differentiate clinically from biliary atresia. The progression of disease is variable and severe cases with liver failure are reported in infancy but majority has persistent liver dysfunction leading to early fibrosis and cirrhosis [6-7]. In our study, all children were diagnosed at an older age due to non-availability of genetic testing in the past except P 8 who was diagnosed at 3-year of age recently. There are no specific clinical and laboratory parameters which leads to diagnosis of NSC and that was another factor for delayed diagnosis though they had history of presentation in the neonatal life or early infancy. Neonatal cholestasis is the major presentation with hepatosplenomegaly and early development of fibrosis and portal hypertension is a feature in these children [8]. In our study, all of them had neonatal cholestasis and portal hypertension which is similar to international literature.

Biochemical features of deranged liver function with cholestasis and markedly elevated GGT in the make it really challenging to differentiate from biliary atresia. The cholangiogram has very important role in differentiating between NSC and biliary atresia and even few reports mentioned about laparotomy for kasai procedures and were found to have patent bile ducts and finally diagnosed as NSC on molecular testing [9]. Only three of our patients had cholangiogram which were reported as patent biliary channels. Liver biopsies findings are ductular proliferation, portal tract inflammation, bridging fibrosis, and most patients developed biliary cirrhosis. Other histologic findings included ductal bile plugs, variable ectasia of perihilar bile ducts, absence of bile ducts in areas of fibrosis, and chronic cholestasis. All of our patients had histological findings including porto-septal fibrosis, bile ductular proliferation and intrahepatic cholestasis similar to international literature [8,9].

Neonatal sclerosing cholangitis is mainly associated with DCDC [2,9] gene mutation resulting in manifestation of progressive liver disease and biliary cirrhosis. DCDC2 is expressed in many huma organs including cholangiocytes and down regulation of DCDC [2] leads to abnormal signaling from cilia as a result of toxic exposure of bile salts and thereby resulting in blebbing of cholangiocytes leading to inflammation, proliferation and fibrosis. Our six patients of neonatal sclerosing cholangitis had DCDC [2,10] mutation, two were compound heterzygous and four were homozygous and product of consanguineous marriage. All were born full term with normal birth weights and had clinical features of neonatal cholestasis, acholic stools, failure to thrive, pruritis and four of them developed severe early portal hypertension. However liver biopsy revealed porto-septal fibrosis, bile ductular proliferation and intrahepatic cholestasis. None of them had renal, neurological, olfactory or hearing involvement. Kabuki and ILVASC (Ichthyosis, Leukocyte Vacuolation, alopecia and Sclerosing Cholangitis) resulting from claudin-1 deficiency (CLDN1) are associated syndromes with NSC [10,11]. Two of our patients had severe pruritis, Ichthyosis, deranged liver enzyme, diffuse alopecia with thin sparse hair without any leucocytes vacuolation.

Table 2: Genetic mutations in families with neonatal sclerosing cholangitis

Families	Patients	GENE	Zygosity	CODON	Mutations
Family 1	Patient 1	*DCDC2*	Homozygous	c.203C>G; p.Pro68Arg	Novel
	Patient 2	*DCDC2*	Homozygous	c.203C>G; p.Pro68Arg	Novel
Family 2	Patient 3	*DCDC2*	Homozygous	deletion in ch 6 encompassing exon 9	Novel
	Patient 4	*DCDC2*	Homozygous	deletion in ch 6 encompassing exon 9	Novel
Family 3	Patient 5	*DCDC2*	Homozygous	c.559C>T; 1 p.Leu187Phe	Novel
	Patient 6	*DCDC2*	Homozygous	c.559C>T; p.Leu187Phe	Novel
Family 4	Patient 7	*DCDC2*	Homozygous	c.559C>T; 1 p.Leu187Phe	Novel
Family 5	Patient 8	*DCDC2*	Homozygous	c.559C>T; 1 p.Leu187Phe	Novel
Family 6	Patient 9	*CLDN1*	Homozygous	c.461dup; p.Val155Serfs* 186	Novel
Family 7	Patient 10	*CLDN1*	Homozygous	c.461dup; p.Val 155Serfs*186	Novel

Neonatal sclerosing cholangitis is a progressive disease and invariable require liver transplant. Supportive management with fat soluble vitamins and ursodeoxycholic acid using its cholretic effect thereby reducing damage to cholangiocytes and improving liver functions. All our patients are stable on supportive therapy except two children who required liver transplant because of uncontrolled portal hypertension and hepatopulmonary syndrome [11].

Limitations of our study was its retrospective nature, small sample size due to rarity of disease, however, this study highlighted one of the important differential in high GGT cholestasis.

CONCLUSION

Neonatal sclerosing cholangitis is a rare condition of genetic cholestasis presenting with jaundice, pale stools, severe liver disease and elevated GGT in highly consanguineous families. These children develop early portal hypertension but with medical measures majority remain stable but invariably require liver transplant as a definitive treatment. Molecular testing can make a difference among other conditions presenting with similar symptoms.

Consent to Publication: Author(s) declared taking informed written consent for the publication of clinical photographs/material (if any used), from the legal guardian of the patient with an understanding that every effort will be made to conceal the identity of the patient, however it cannot be guaranteed.

Authors Contribution: Author(s) declared to fulfill authorship criteria as devised by ICMJE, the authors confirm contribution to the paper as follows. **SSBQ**: Author, **AS**: Proofread collection, **ZF, AA, NA**: Data Collection, **SI**: Geneticist, Genetic data interpreter, **TF**: Data Collection

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