Genetic Perspectives on Paediatric Liver Transplantation - Indications, Molecular Basis and Prognosis

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Abstract

In recent years, outcomes of Paediatric Liver Transplantation (LT) have greatly improved as reported by various studies from multiple centres in the world. Approximately 1500 liver transplants are performed annually in India, with reports of excellent survival rates surpassing 90% among small children. With availability of treatment options like liver transplantation to even neonates, early detection and management of such cases is gaining importance. There is growing need for clinicians, busy in office practice, to have concise practical information for effective communication with parents regarding basis of disease and its prognosis, need for LT and comprehensive care of such patients. Important concerns still remain such as comprehensive patient care, financial viability of management options. This again indicates importance of raising awareness in medical community about early selection of patients and cost benefit analysis.

Amalgamation of knowledge about genetic diagnosis and its application specifically to cases, where LT is indicated, will help clinicians in guiding the family to timely management of affected members, screening and early detection of other family members and its application in prevention by prenatal diagnosis.

Introduction

In recent years, outcomes of Paediatric Liver Transplantation (LT) have greatly improved due to advancement in organ procurement, preservation and transport methods along with better understanding of host and recipient immune mechanisms [1-4]. Approximately 1500 liver transplants are performed annually in India, with reports of excellent survival rates surpassing 90% among small children [5-7]. In order to improve quality of life of children with liver disease in general, these advancements ought to be accompanied by an increase in awareness that allows clinicians to identify appropriate candidates for LT, and also manage these patients post-transplant.

Difficult judgments must be made to suit the needs of the patient and a lack of timely management could have severe consequences. Patients with inborn errors of metabolism (IEM), detected through newborn screening (NBS) and managed well will have better outcome if LT is needed later. Children with delayed diagnosis and treatment may have systemic or neurological complications which can dilute outcome of LT in terms of benefits and future prognosis.

Indications for LT in paediatric population are extrahepatic and intrahepatic cholestasis, metabolic liver diseases, acute liver failure and tumours. Recent developments in surgical techniques using portion of livers from adult donors has increased the availability of the tissue and improved chances for reducing patient number on waiting list and hence survival outcome [2].

Expanding scientific knowledge on the underlying pathology of diseases and their molecular mechanisms has revolutionized clinical practice, particularly so for diseases with genetic basis [8]. Advances in DNA sequencing has added potential benefits to patients and their families by allowing precise genetic diagnosis and aiding in targeted therapies for specific molecular anomalies. Although these are presently largely available in research and academic domains, expanding these services into clinical practice may soon help address many vexing genetic issues and better therapeutic options.

Molecular Genetics and Liver Disease

The human Genome consists of greater than 21000 genes and contains approximately 3.2 billion base pair nucleotides, with variable degrees of nucleotide positions, such as single nucleotide variants (SNV), to large deletions-duplications i.e. copy number variants (CNV) and although many genes carry mutations, not all of these result in disease [9]. Using array comparative genomic hybridization (aCGH) we can now identify greater number of CNVs. Many of these newer molecular techniques leave us with enormous amount of data and information all of which may not be of clinical relevance [10]. With the use of next generation sequencing (NGS), the entire genome can be analysed which requires software skills and bio-information technology for handling large databases [11]. Finally, there are ethical issues involved with indiscriminate molecular screening and hence it is important to have clinical correlation with appropriate investigations.
of Wilson disease gene by Bull et al in 1993 [12]. Availability of data since completion of Human Genome Project and now genome wide association studies (GWAS), has imparted knowledge about genotype-phenotype correlation of many complex diseases of hepatobiliary system and are paving way to new novel precise therapies. Often genetic studies in association with clinical and biochemical abnormal finding are confirmatory, thereby avoiding need for invasive tests like liver biopsy for diagnosis. In this discussion, we have made an attempt to establish the importance and value of genetic testing in hepatic diseases particularly those that may benefit from LT. It focuses on LT from the perspective of genetic etiologies in the pediatric population, its molecular basis, and different prognostic outcomes in various scenarios. An awareness of such topics may be the key to developing an index of suspicion and ensure timely management.

**Structural and Functional Aspects of Liver Disease**

Although development of liver and biliary system is a complex process, the molecular basis and genetic etiology helps us to understand the pathogenesis of rare inherited liver diseases. The liver develops from the ventral foregut endoderm. Epithelial cells such as hepatocytes and cholangiocytes are formed from endoderm whereas other cells including Kupffer cells, stellate cells, and fibroblasts are derived from mesoderm. Specification of hepatoblasts into hepatocytes and cholangiocytes is coordinated by signals from the adjacent cardiac mesoderm and septum transversum mesenchyme [13]. Epithelial differentiation occurs along a structure called ductal plate formed by primitive hepatoblasts. Altered development of ductal plate leads to malformations which present at early ages as atresia of biliary structures and is sometimes associated with anomalies in other organs such as heart, kidneys, pancreas or nervous system [14].

Alagille syndrome is an autosomal dominant disorder with paucity of intrahepatic ducts causing cholestasis associated with cardiac, skeletal and ocular abnormalities. It is caused by mutations in JAG1 and NOTCH2 genes which are important in signals for ductal morphogenesis [15,16]. Congenital hepatic fibrosis is a histopathologic diagnosis which refers to a developmental disorder characterized by defective remodeling of the ductal plate, progressive fibrosis of the portal tracts. It is associated with ciliopathies (disorders of the primary cilia) and renal involvement in conditions like polycystic kidney disease, nephronophthisis, Meckel Gruber syndrome, Jeune syndrome collectively referred to as the hepatorenal fibrocystic diseases [14,16].

Analysis of genes in patients with cholestatic disease and livers of postnatal forms of biliary atresia have revealed anomalies in the expression of several genes involved in morphogenesis, fibrogenesis, transcriptional regulation and cell signaling. This has given better insights into the etiology of these diseases. However, role of some of these genes in pathogenesis remains uncertain [17, 18].

The involvement of the liver in the form of hepatomegaly, liver failure or cirrhosis is a feature of many metabolic disorders as the liver is an organ central to carbohydrate, protein, fat, vitamins and trace element metabolism. Many IEMs can be detected early by tandem mass spectroscopy based newborn screening. Genetic diagnosis has led to accuracy, detection of affected as well as carrier members in the family, prenatal diagnosis and has stimulated research for novel treatments in IEM.

Mature hepatocytes have been shown to contribute to liver regeneration via proliferation and hypertrophy. They exhibit high plasticity and ability to acquire progenitor cell phenotype [19]. This further led to development of newer cell based therapies like liver cell transplantation (LCT) wherein cells infused via portal vein integrate into recipient parenchyma, proliferate and correct functional deficiency in some IEMs.

**Indications of Liver Transplantation in Paediatric Patients and Their Genetic Basis**

The list of conditions which can be treated by LT is growing [2,4,13]. Biliary atresia is the most common indication of paediatric LT followed by metabolic diseases, autoimmune conditions, familial cholestatic diseases and acute liver failure [1,2,4]. The genetic basis of many diseases is increasingly becoming clear from growing clinical evidence and increase in the use of GWAS using NGS based multi-gene panel [17,20,21] and helps in confirmation of etiology. Careful selection of patients for LT is important and depends on many factors such as primary etiology, multiorgan involvement, response to available drug treatment and its impact on growth and development (Table 1).

Apart from conditions listed in the table, other causes related to drug toxicity, autoimmune conditions may have multifactorial basis including genetic, however these are beyond the scope of present discussion.

**Metabolic Liver Diseases Amenable to LT**

Some metabolic diseases can be completely cured by LT where as in others it helps in acute liver crisis or improves the functional deficiency and thereby prognosis. LT is usually considered when medical management fails, has severe side effects, in the event of acute crisis or possibility of hepatic malignancy.

Some diseases lead to chronic liver disease, cirrhosis or malignancy where LT helps in cure as in following disorders:

**Tyrosinemia type I:** Presence of succinylacetone in newborn blood detected by tandem mass spectroscopy (TMS) is pathognomonic for tyrosinemia type I. Untreated infants present either with early onset severe liver involvement or later with liver dysfunction and renal tubular dysfunction associated with growth failure and rickets. There is an increased risk of hepatocellular carcinoma. It results from deficiency of enzyme fumarylacetoacetase (FAH). The diagnosis is established by typical biochemical findings, elevated plasma concentrations of tyrosine, methionine, and phenylalanine and elevated urinary concentration of tyrosine metabolites and/or by identification of mutations in FAH gene. Nitisinone treatment should begin as soon as the diagnosis is confirmed along with dietary management with controlled intake of phenylalanine and tyrosine.

**Glycogen storage diseases (GSDs):** The clinical manifestations vary with various subtypes and age of onset. GSDs predominantly affecting liver are types 0, 1, 3, 4, 6, 9 and 11. They may present with fasting hypoglycemia, hepatomegaly, and growth retardation. Some types (III, IV, IX) may be associated with liver cirrhosis. Extrahepatic manifestations may be present like as renal dysfunction in type I, myopathy (skeletal and/or cardiomyopathy) in types III and IV. Presence of diastase-sensitive PAS-positive glycogen staining in liver biopsies is helpful in making diagnosis. Differentiation between subtypes is important for optimum management, and requires diagnosis by enzyme assays in the liver and other tissues and mutation analysis. LT is
indicated in those with progressive liver damage or malignant transformation of adenomas into hepatocellular carcinoma.

<table>
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Table 1: Lists indications of LT associated with possible genetic etiologies.

**Abbreviations:** AD- Autosomal Dominant; AR- Autosomal Recessive; AAT- Alpha1antitrypsin; LDL- low density lipoprotein, ARPKD- Autosomal Recessive Polycystic Kidney Disease; OMIM- Online Mendelian Inheritance In Man.

**Wilson disease:** It is a disorder of copper metabolism that can present with hepatic, neurologic, or psychiatric disturbances or combination of these. The diagnosis is established by correlation of clinical findings with biochemical findings such as low serum copper, ceruloplasmin concentrations and increased urinary copper excretion or pathogenic mutation in ATP7B gene. Treatment is by copper chelation agents which increase urinary excretion of copper.

**Hereditary fructose intolerance (HFI):** In case of dietary exposure to fructose, sucrose, or sorbitol, untreated HFI is characterized by hypoglycemia, lactic acidemia, hypophosphatemia, hyperuricemia, hypermagnesemia, hyperalaninemia. Patient may present with vomiting, abdominal distress, growth failure, sometimes acute liver failure. The diagnosis established by biallelic pathogenic variants in ALDOB gene or deficient hepatic fructose 1-phosphate aldolase (aldolase B) activity on liver biopsy. Sometimes uncontrolled disease with liver involvement may need LT.

**α1-Antitrypsin deficiency:** The most common manifestation of liver disease is hyperbilirubinemia and raised serum aminotransferase levels at early age. Diagnosis relies on demonstration of low serum concentration of AAT and either detection of functionally deficient AAT protein variant by protease inhibitor typing or detection of pathogenic variants in SERPINA1 gene. Vitamin E therapy improves liver function in few specific genotypes and in children with cholestasis, also helps to prevent oxidative damage to the lungs.

In some metabolic disorders, LT is done in case of acute decompensation or to prevent metabolic disturbances, improve prognosis and quality of life of patients such as in following diseases:

**Urea cycle defects (UCDs):** They result from inherited deficiencies in any one of the six enzymes or two transporters of urea cycle pathway. Plasma concentration of citrulline helps to discriminate between the proximal and distal urea cycle defects. Plasma concentration of arginine is markedly elevated in arginase deficiency. Molecular genetic testing is the primary method of diagnostic confirmation for all eight UCDs and has supplanted measurement of enzyme activity. UCD deficiency is inherited in an X-linked manner and other UCDs are inherited in autosomal recessive manner. Drug treatment (sodium phenylacetae and sodium benzoate), arginine, citrulline supplementation and dietary protein restriction helps in improving neurological outcome. The transplanted liver provides sufficient enzymatic activity to correct the deficiency and removes the risk of metabolic decompensation and need for dietary protein restriction.

**Organic academia:** They are caused due to defects in the catabolic pathways of branched-chain amino acids, also accumulation of organic acids derived from lysine, and dicarboxylic acidemias associated with defective fatty acid degradation. Definitive diagnosis is usually established by identifying and measuring specific organic acids in body fluids (blood, urine), by enzymatic assay and by identification of the mutant gene. NBS helps in detection and early therapeutic management by dietary restriction of specific amino acids. In some patients with propionic acidemia and methyl malonic academia LT may be done in case of frequent metabolic decompensations, uncontrollable hyperammonemia, and/or poor growth.

**Citrin deficiency:** It can manifest in infants as neonatal intrahepatic cholestasis, in older children as failure to thrive and dyslipidemia and in adults as recurrent hyperammonemia with neuropsychiatric symptoms in citrinulinemia type II. Beyond age one year, many children develop a protein-rich and/or lipid-rich food preference and aversion to carbohydrate-rich foods. Clinical features include growth restriction, hypoglycemia, pancreatitis, severe fatigue, anorexia, and impaired quality of life. They have dyslipidemia, increased lactate-to-pyruvate ratio, considerable deviation in tricarboxylic acid cycle metabolites, increased ammonia, citrulline and serum concentration of pancreatic secretory trypsin inhibitor, biallelic pathogenic variants in SLC25A13 gene. Diet supplementation with fat-soluble vitamins and use of lactose-free, medium-chain triglyceride enriched diet, administration of sodium pyruvate may improve growth and delay need for LT. It prevents hyperammonemic crises, corrects metabolic disturbances.
Crigger-Najjar type I: It is caused by deficient uridine diphospho-glucuronate glucuronyltransferase (UDPGT) activity and defective glucuronidation producing congenital unconjugated hyperbilirubinemia. UGT1A1 is the primary UDPGT isoform and complete absence of its activity causes Crigger-Najjar type I. The diagnosis is based on the early age of onset and bilirubin elevation in the absence of hemolysis. Definitive diagnosis is established by measuring hepatic glucuronidyltransferase activity in a liver biopsy specimen and/or pathogenic mutation in UGT1A1 gene.

Familial hypercholesterolemia: It is characterized by severe elevation of LDL cholesterol levels that lead to atherosclerotic plaque deposition in the coronary arteries and proximal aorta at an early age, leading to an increased risk for cardiovascular disease. Approximately 70%-95% patients have a heterozygous mutation in one of three genes (APOB, LDLR, PCSK9). Management of diet and physical activity is recommended at an early age. Statins are the preferred treatment. Liver transplantation has been used in rare circumstances.

Primary oxalosis Type I: It is caused by a deficiency of the liver peroxisomal enzyme alanine glyoxylate aminotransferase (AGT) which catalyzes the conversion of glyoxylate to glycine. Glyoxylate is converted to oxalate forming insoluble calcium salts that accumulate in the kidney and other organs and may lead to end stage renal disease. Diagnosis is made by elevated oxalate to creatinine ratio in urine, elevated plasma oxalate concentration, liver biopsy to assay the activity of AGT and/or detection of mutation in AGXT gene. Initial therapies are based on prevention and treatment of renal stones and diet restriction.

Liver Transplantation Outcome and Prognosis

Rationale behind organ allocation was improved and there was evolution of the strategies based on data derived from the Studies of Pediatric Liver Transplantation research group. Pediatric end-stage liver disease score (PELD) was created, using bilirubin, INR, serum albumin, age > 1 year, and growth failure to predict waiting list mortality [22]. Spada et al. have discussed various state of the art surgical techniques in pediatric LT, patient preparation, donor selection and complications [2].

Early detection of metabolic diseases through newborn screening has improved patient care. As mentioned in Table 1, many diseases amenable to be treated by LT may have genetic basis which can be confirmed by doing specific gene analysis using Sanger sequencing technique. Confirmed diagnosis helps in prognostication of the disease and its impending course of complications may be anticipated. Whole exome analysis has improved screening of many genes in relatively small period and with less expenses. Evolving data of genotype-phenotype correlation of these diseases is helping to screen these patients early for complications. Discussion with patient families, regarding role of LT, can be done well in advance for better preparations.

Growth failure is common in chronic liver disease while neurological impairment, involvement of other systems and organ failure are important in acute decompensation due to metabolic disease. These patients require adequate calories, protein and nutrition supplements to achieve adequate growth. Carefully managed nutritional support considering primary disease, before transplant, is associated with improved surgical and neurodevelopmental outcomes [23,24].

Children with chronic liver disease should undergo neurodevelopmental assessment and early intervention to minimize long-term cognitive delays [23,25]. Biliary atresia is the most common indication for LT, and of children transplanted at <1 year of age, studied by McDiarmid et al [1], 65.6% had biliary atresia. In intrahepatic cholestatic diseases, LT is indicated to eliminate severely debilitating symptoms like pruritus. Children affected by these diseases are at high risk of development of liver malignancies [3,25]. Metabolic diseases are the second most common indication for LT with many causing structural damage, fibrosis and cirrhosis. Good outcomes have been achieved in urea cycle disorders, especially ornithine transcarbamoylase deficiency [26,27]. LT in IEMs requires careful perioperative management and some disorders require long-term dietary restriction, medical management, and monitoring of metabolites [27,28]. Better results for these complex disorders can be achieved with disease and patient-specific strategies using a multidisciplinary approach towards primary disease management with the aim of optimum growth and neurological development. Although quality of life is improved, patients remain at risk of severe extrahepatic disease complications [28]. In primary oxaluria, consideration of LT before end-stage renal disease restores enzyme activity and decreases ongoing oxalate synthesis [29] and combined liver-kidney transplantation is preferred over isolated renal transplant. The etiology of acute liver failure (ALF) remains elusive in almost half of affected children. Inherited mitochondrial and fatty acid oxidation disorders are some of the etiological factors in patients with idiopathic ALF and impaired energy metabolism [30,31]. However, these disorders involve multiple systems and careful selection of patients is important considering long term risks versus benefits. In neonatal hemochromatosis, LT is effective for reducing hepatic iron load but metabolic defect remains [32]. Liver cirrhosis with severe portal hypertension develops in about 25% of patients affected by cystic fibrosis, hence LT should be considered before the development of end-stage liver failure while pulmonary function is still preserved [33].

Good outcome in children has been achieved with use of living donor [4]. The use of split livers from deceased donors and partial grafts from living donors [34,35] as well as heterozygote parent [36] has yielded encouraging results in terms of graft viability. The use of living donation in pediatric transplantation is shown to be associated with good results in the child, as well as being generally safe for the donor [37]. In India, there are hardly any deceased donors used for transplanting children. This is due to scarce awareness amongst pediatric intensivists about organ donation in brain dead children and also not splitting adult deceased donor livers which could potentially benefit an adult and a child.

LCT has shown moderate and transient benefit in animal models as well as few clinical settings, but more studies are needed further. It has been shown to be especially useful in metabolic diseases as a bridge to transplantation [38,39].

Summary

Paediatric liver transplantation is a state-of-the-art treatment method with good success rates in most metabolic liver diseases. It is a life-saving intervention for children with end stage liver disease. The paediatrician plays a key role in the referral process as well as in the optimization of patient care both pre and post-transplant.

Citation: Snehal M, Merchant R, Nagral A, Mirza D (2019) Genetic perspectives on paediatric liver transplantation - Indications, molecular basis and prog-
Timely referral is possible with early precise diagnosis. Identification of appropriate candidate for transplant involves assessment of severity of primary disease, comorbidities affecting transplant survival and confirming that effective alternative treatment options have exhausted. Newborn screening for all inborn metabolic diseases has still not been widely accepted but is gaining momentum [40]. Effective dietary management and medical treatment of metabolic diseases helps in getting better outcome of LT. Post-transplant care in pediatric patients also needs multidisciplinary management regarding nutrition, growth and development, care of infections and immunization.

In India, as a result of scarcity of facilities, lack of awareness and delayed diagnosis, this intervention has not helped many patients. Recent advances in molecular diagnostics are rapidly changing our outlook towards molecular basis of disease and accordingly therapeutic. With the growing application of diagnostic methods in day to day practice, we can gather evidence about genotype-phenotype correlation and prognosis of patients and thus help in optimizing resources of treatment. Genetic diagnosis helps in confirmation of precise etiology which helps patients and their families understand the nature of the disease and its prognosis. Genetic counseling helps families to understand recurrence risk and recognize at risk family members. This is also important for prenatal counseling and management. Research into therapies like hepatocyte transfer, especially in patients awaiting LT and definitive molecular targets to genetic diseases is yet to reach the stage of wide practical application but holds promising outcomes.

References

19. Online Mendelian Inheritance in Man, OMIM.


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Journal of Addiction & Addictive Disorders
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