Alpha-1 antitrypsin deficiency among Indian children with liver disorders

Rajeev Khanna, Seema Alam, Rana Sherwani,* Shivali Arora,** N K Arora,** Ashraf Malik

Departments of Pediatrics and *Pathology, JNMC, AMU, Aligarh 202002; and **Department of Pediatrics, All India Institute of Medical Sciences, New Delhi 110 029

Aims: To determine the frequency of alpha-1 antitrypsin (AAT) deficiency in children with chronic liver disease (CLD) and neonatal cholestasis syndrome (NCS).

Methods: All children with NCS (n=23) or CLD (n=35) attending the Pediatric Gastroenterology Clinic between November 2003 and July 2005 were screened for AAT deficiency using phenotyping through isoelectric focusing of plasma. Results: Of the 58 children studied, 57 had normal PiMM phenotype. One child with CLD had the M type of normal variant. None of the patients had the abnormal phenotype PiZZ. Conclusion: AAT deficiency is infrequent among children with CLD and NCS in our region. [Indian J Gastroenterol 2006;25:191-193]

Alpha-1 antitrypsin (AAT) deficiency with PiZZ phenotype is an autosomal recessive disorder with frequency of 1 in 1600 to 2000 live births in Europe.1,2 In Southeast Asia, the estimated numbers of carriers of AAT deficiency (PiMZ phenotype) and AAT-deficient persons (PiZZ phenotype) are 1,600,000 and 11,000, respectively.3

The disorder is associated with 85%-90% reduction in serum concentration of AAT. The mutant AAT molecules are retained in the hepatocyte endoplasmic reticulum and are hepatotoxic. The retained AAT appears as periodic acid-Schiff (PAS)-positive, diastase-resistant eosinophilic inclusions in the periportal hepatocytes.4 Nearly 10%-15% of individuals with PiZZ AAT phenotype develop liver injury, which may take the form of neonatal cholestasis syndrome (NCS), hemorrhagic disease of the newborn, compensated or decompensated chronic liver disease (CLD) during childhood, asymptomatic derangement of liver function tests, or hepatocellular or cholangiocellular carcinoma in adults.5

Several methods are available for the diagnosis of AAT deficiency. Serum AAT levels can be measured using immunoassays (radial immunodiffusion or nephelometry); however, these may be falsely normal during periods of acute inflammation. Phenotyping of AAT enzyme in plasma is possible using agarose gel electrophoresis, acid-starch gel electrophoresis or isoelectric focusing in polyacrylamide gel. These techniques, based on differential electrophoretic mobility of different variants of AAT protein, are not affected by inflammatory conditions and can detect heterozygous AAT states. However, these methods require considerable experience and may fail to detect persons with null variants (complete absence of AAT).6,7

AAT genotyping using polymerase chain reaction and sequencing can detect specific mutations in the AAT gene, and can also detect null alleles; however, it is expensive and is available only in research laboratories.8,9 Finally, liver biopsy may show presence of eosinophilic globules in hepatocytes on PAS-diastase staining. However, these globules are usually not seen before 3 months of age10 and may be present in some other disease conditions too.

In studies from the West, AAT deficiency has been reported in 0.5%-2% of children with CLD11,12,13 and 13%-18% of those with NCS.13,14,15 There is some corresponding Indian data.16-20

Methods

All children with CLD and infants with NCS presenting to the Pediatric Gastroenterology Clinic, J N Medical College, Aligarh, between November 2003 and July 2005 were included in the study. CLD was defined as the presence of clinical features suggestive of liver dysfunction or of cirrhosis for more than 3 months, supported by biochemical abnormality (abnormal transaminases, raised serum bilirubin, low serum albumin or prolonged prothrombin time), or histological evidence of chronic liver injury. NCS was diagnosed in infants less than 6 months of age presenting with jaundice, dark-colored urine or acholic stools starting in the neonatal period (first four weeks of age) or later up to six months of age, with conjugated hyperbilirubinemia (direct bilirubin more than 2 mg/dL or more than 15% of total bilirubin) persisting beyond 14 days of life.21 The cut-off levels for AST and ALT of our laboratory are 20 and 15 IU/L, respectively. Values at presentation to the Pediatric Gastroenterology clinic were considered for analysis.
HBsAg and anti-HCV antibodies were tested in all cases. Due to financial constraints, serum ceruloplasmin and TORCH screening were done in only 22 and 12 cases each of CLD and NCS, respectively.

Percutaneous liver biopsy was examined for the presence of PAS-positive diastase-resistant globules in periportal hepatocytes, wherever possible. AAT phenotyping was done by isoelectric focusing of plasma using the Phast System (Amersham Biosciences, Sweden) in the Pediatric Gastroenterology division at the All India Institute of Medical Sciences, New Delhi. The gels were stained with Phast Gel Blue R (commassie blue stain) and Phast Gel Silver kit (silver stain), and bands 4 and 6 were looked for. During each experiment, serum specimens from persons known to have normal PiMM and abnormal phenotype were run for comparison.6,7

The institutional ethics committee of the J N Medical College, Aligarh cleared the study.

Results

The baseline characteristics of 35 children with CLD and 23 with NCS seen during the study period are summarized in the Table. None of the CLD patients had Kayser-Fleischer ring on slit-lamp examination or any congenital anomaly on ultrasonography. Transaminase levels were abnormal at the time of first presentation in 25 of 35 (71%) patients with CLD and 20 of 23 (87%) patients with NCS. Prothrombin time was prolonged in 69% (mean [SD] INR 1.93 [0.86]) of CLD and in 60.9% (INR 2.00 [1.03]) of NCS cases. The three cases with serum ceruloplasmin below 200 mg/L were between 6 to 9 years of age.

Percutaneous liver biopsy was not possible in 13 children with CLD and 11 with NCS, either due to refusal by parents or due to persistent coagulation disturbance. Of the 22 children with CLD, 13 had chronic hepatitis, 5 had cirrhosis, 2 had fibrosis, and one each had storage disorder and vascular malformation. The etiology could be ascertained in 10 cases of CLD; 4 were HBV infection, 3 Wilson’s disease, and 1 each HCV infection, glycogen storage disease and vascular malformation. Of the 12 infants with NCS, biopsy showed neonatal hepatitis in 8, extrahepatic biliary atresia in 2, and cholestasis and cirrhosis in one infant each. None of the biopsies in either group of patients showed staining for AAT.

On isoelectric focusing, 57 cases had the normal PiMM phenotype. One child with CLD had the MIE type of normal variant. He was an eight-years-old boy who was seronegative for hepatitis B and C, had normal 24-hour urinary copper and had cirrhotic changes on liver biopsy. Deficiency phenotype PiZZ was not seen in any case.

Discussion

In our study, most patients had the normal PiMM AAT phenotype and one had a normal variant MIE, which is known to be not associated with deficient AAT levels in serum or with lung or liver disease.22 None of the patients had AAT-deficient phenotypes.

The prevalence of AAT deficiency varies in different geographical regions, being the highest in northern and western Europe with a mean gene frequency of Z allele of 0.0140 among Caucasians.3 In North America, the average gene frequency is 0.0092, and in Australia and New Zealand, it is 0.0151.3 Cohorts from Japan, China and South Korea have been reported to have the Z allele gene in 0.0002, 0.0 and 0.0061, respectively.3 In the remaining parts of Asia, estimates indicate a low gene frequency of Z allele – ranging from 0.0036 in Southeast Asia to 0.0056 in the Middle East.3

Several studies have suggested AAT deficiency to be an important cause of NCS and CLD in children. In three studies, AAT deficiency was found to be responsible for 1 of 196 (0.5%), 3 of 152 (2%) and 8 of 464 (1.7%) cases with CLD, respectively.11,12,13 Similarly, among infants with NCS, AAT deficiency was detected in 67 of 409 (16.4%), 7 of 54 (13%) and 189 of 1086 (17.4%) cases, respectively.13,14,15 Of these studies, three used phenotyping11,13,14 and one used serum AAT level measurement.12 In comparison, in India, AAT deficiency has been found in only 1%-4% of children.
with CLD and NCS.16-20 None of these reports included the details of diagnostic test used. In a study from Bangladesh,23 one of 62 (1.6%) infants with NCS had AAT deficiency, based on liver biopsy assessment and serum AAT level measurement.

The slightly higher detection rate of AAT deficiency in the previous Indian studies than the present study could either be due to the usage of different reference methods for diagnosis or area-wise variability of the prevalence.

We conclude that the prevalence of alpha-1 AT deficiency phenotype in this part of our country (western Uttar Pradesh) is very low.

References