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Low incidence of alpha-1-antitrypsin deficiency in Iranian patients with neonatal cholestasis

LIVER

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Original Article

ABSTRACT

Background/Aims: There is little data concerning the incidence of alpha-1-antitrypsin"(AAT) deficiency, the most common genetic cause of liver disease, among children with neonatal cholestasis in Iran. Thus, this study was performed to analyze AAT deficiency in this group of patients.

Materials and Methods: DNA samples from patients with neonatal cholestasis were investigated for Pi S and Pi Z alleles, using polymerase chain reaction–restriction fragment length polymorphism.

Results: Thirty patients with neonatal cholestasis were enrolled. Among those who underwent biopsies, the results revealed neonatal hepatitis in 19, bile duct paucity in 1, steatohepatitis in 1, bile duct proliferation in 1, cirrhosis in 2, fibrosis in 2, and extrahepatic biliary atresia in 1 patient. No mutant allele was found in any patient.

Conclusion: The incidence of AAT deficiency is very low in Iran; therefore, screening for AAT is not recommended for patients with neonatal cholestasis in Iran.

Keywords: Alpha-1 antitrypsin deficiency, neonatal cholestasis, biliary atresia

INTRODUCTION

Alpha-1-antitrypsin(AAT) is a protease inhibitor that is produced by hepatocytes, lung epithelium, phagocytes, and small intestinal and renal tubular cells (1). The main function of the protein is inhibition of proteases, particularly neutrophil elastase. The gene (SERPINA) encoding AAT is located on the long arm of chromosome 14 (2). More than 100 variants have been detected. The most common mutant alleles are Pi Z and Pi S. Pi Z results from the substitution of lysine for glutamate at position 342; Pi S results from the substitution of glutamic acid for valine at position 264 and correlates with milder reduction in serum AAT levels compared with the Z allele (3). Mutant variants are accumulated in hepatocytes, leading to inflammation, fibrosis, and cirrhosis. AAT deficiency, an autosomal recessive metabolic disorder with co-dominant expression, is the most common genetic cause of liver disease in children and infants, manifesting as neonatal cholestasis, chronic hepatitis, cirrhosis and acute liver failure (4,5). Diagnosis can be established y measuring the serum levels of AAT, phenotyping by isoelectric focusing, and genotyping. Phenotyping is considered as the gold standard for diagnosis, where the products migrate depending on their charge in the gel (6).

In this study, we aim to investigate the incidence of AAT deficiency among children with neonatal cholestasis by measuring serum levels of AAT and genotyping.

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MATERIALS AND METHODS

Thirty children admitted to the Pediatric Gastroenterology department at the Children's Medical Center for neonatal cholestasis, from November 2006 to February 2012, were enrolled in the study. After obtaining written consent from parents, the patients underwent clinical and paraclinical work-ups including physical examination of the liver, liver function tests, complete blood count, ultrasound of the liver, HIDA scan, serum AAT levels, and genotyping of AAT. Liver biopsy was performed for the presence of PAS-positive diastase-resistant globules in periportal hepatocytes.

Peripheral blood (5 mL) was collected from each patient and stored in ethylene-diaminetetraacetic acid tubes. DNA was extracted using the Qiagen kit. Subsequently, DNA samples of the patients were investigated for mutant variants PiZ, and PiS (E342K and E264V mutations) using polymerase chain reaction–restriction fragment length polymorphism. Electrophoresis was performed on 8% agarose gel for the separation of products.

RESULTS

The demographic features of the patients are shown in Table 1. Thirty patients were investigated, of which 2 were excluded due to hypothyroidism and positive sweat test. All patients were negative for mutant alleles. Liver biopsy was not performed in 2 subjects due to coagulation disturbances. The results of the biopsy are shown in Table 2.

Transaminase, alkaline phosphatase, gamma-glutamyl transpeptidase, international normalized ratio, and total and conjugated bilirubine levels were elevated in 27, 12, 18, 14, and 27 patients, respectively. One patient had a low levels of AAT and presented with jaundice a month after birth. HIDA scan was conducted in 8 patients; the results showed lack of liver uptake in 6 patients and biliary obstruction in 2 patients. None of the biopsies showed AAT after staining.

DISCUSSION

This study intended to determine AAT deficiency among patients with neonatal cholestasis according to serum AAT level and AAT genotype. There are several studies concerning the correlation between AAT deficiency and liver diseases from different geographical regions. Our findings show that the incidence of AAT deficiency is very low. None of the patients had deficient AAT genotype. One patient had low serum AAT levels. Some conditions reduce serum AAT levels, including hepatic failure, nephrotic syndrome, malnutrition, cystic fibrosis, and respiratory distress syndrome (7).

AAT deficiency is more prevalent in Europeans, with a prevalence of 1 in 2500 in western countries (8), but it can affect all races world wide. The highest prevalence of the Z allele was identified in European countries with a mean gene frequency of 0.0153 in northern Europe, while the frequency is lower in Southeast Asia and the Middle East (0.0036 and 0.0056 respectively) (9). In the report by Tan et al. (10), a low incidence of AAT deficiency (0.7%) was detected among 96 Filipino patients with neonatal cholestasis. Similar results have been reported in Asian countries (11-13). In comparison, a high incidence of AAT deficiency (13%-17.4%) has been reported in patients with neonatal cholestasis in western studies (14-16).

Table 1. Demographic features of patients with neonatal cholestasis (N=28)

Mean Age (Range)	Female/Male	Consanguineous parents
1.27 years (33 days-8 years)	13/15	19

Table 2. Liver biopsy findings in patients with neonatal cholestasis (N=26)

Finding	N (%)
Neonatal hepatitis	19 (73.1)
Bile duct paucity	1 (3.8)
Steatohepatitis	1 (3.8)
Bile duct proliferation	1 (3.8)
Cirrhosis	2 (7.7)
Fibrosis	2 (7.7)

Table 3. Results of previous Iranian studies that performed molecular analysis of AAT

Author/year	Number of patients	Frequency of mutant allele
Jowkar et al./2013	126	0%
Lotfi et al./2005	307	16.12%

Our observations are in accordance with previously published data from Iran stating the low incidence of AAT deficiency among children with liver diseases (17,18) (Table 3).

In conclusion, our data showed that the incidence of AAT deficiency in patients with neonatal cholestasis in the largest pediatric center in Iran is very low. Further studies in different regions are needed to confirm the results of this study.

Ethics Committee Approval: Ethics committee approval was received for this study.

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

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