

Original Article

Low Incidence of α -1 Antitrypsin Deficiency among Babies with Prolonged Jaundice

Zahra Jowkar MS¹, Bita Geramizadeh MD^{1,2}, Sara Fanai MD¹, Mitra Mirzai MS¹, Seyed Mohsen Dehghani MD³, Mahmoud Haghghat MD³, Naser Honar MD³, Seyed-Ziyaodin Tabei MD¹

Abstract

Background: α -1 antitrypsin (AAT) deficiency is one of the most important genetic causes of childhood liver diseases in some parts of the world, but its geographic distribution is highly variable. There are many reports from Asian countries such as India, the Philippines, and China which show a very low incidence of this disease. However few studies exist from Iran regarding this genetic deficiency as the cause for prolonged neonatal jaundice. In this study we attempt to investigate the possible role of AAT deficiency as a cause of prolonged neonatal jaundice in the largest pediatric referral center of Southern Iran.

Methods: We included 126 neonates with the clinical diagnosis of neonatal cholestasis in this study. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was performed on the extracted DNA from their blood samples. DNA sequencing confirmed the results of the PCR-RFLP tests.

Results: All patients were genetically normal regarding level of AAT, i.e., all were MM homozygotes.

Conclusion: AAT deficiency is a rare disease in Iran and is not a major cause of neonatal cholestasis in this country.

Keyword: α -1 antitrypsin deficiency, neonatal cholestasis, south of Iran

Cite this article as: Jowkar Z, Geramizadeh B, Fanai S, Mirzai M, Dehghani SM, Haghghat M, et al. Low incidence of α -1 antitrypsin deficiency among babies with prolonged jaundice: *Arch Iran Med.* 2013; **16**(1): 23 – 24.

Introduction

α -1 antitrypsin (AAT) is a 52 KD glycoprotein mostly secreted by hepatocytes, lung epithelium, and phagocytes.¹

AAT deficiency is one of the most common hereditary disorders worldwide but its prevalence varies among different countries.² This disease has been first described by Laurell and Erikson in 1963. Since then, studies in Caucasians suggest that AAT deficiency is one of the most common etiological factors associated with chronic liver diseases in children.³

The frequency of Z- or S-deficient alleles in Western countries varies from 0.004% to 0.1%. Patients that have a PiMZ and PiZZ phenotype usually present with elevated liver enzymes and cholestasis as neonates.¹ The reported incidence of AAT deficiency in Western countries is about 10% (5% – 15%).⁴ There are few studies regarding the incidence of the above mentioned genotypes in neonatal liver diseases from Iran. We have performed this study to evaluate the importance of AAT deficiency as a cause of prolonged neonatal jaundice in Iran.

Patients and Methods

Patients

The study population consisted of 126 neonates (under the age

of 1 year) who were admitted to Namazi Hospital, affiliated with Shiraz University of Medical Sciences, with a clinical diagnosis of prolonged jaundice. Prolonged jaundice was defined as clinically apparent jaundice in any infant beyond the first 14 days of life.⁵

Methods

Blood samples were taken and stored at -70°C prior to DNA isolation. DNA was isolated by a high yield DNA isolation kit (Qiagen, USA), after which polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was performed by using the following primers:

PFZ: 5' -ATAAGGCTGTGCTGACCATGCT- 3'
PRZ: 5' -TTGGCTGGGATTCAGGACTTTTC- 3'

The Taq-1 restriction enzyme was also used. Subsequently, electrophoresis was performed on a 3% agarose gel. Normal alleles were digested by the Taq-1 enzyme into two, 157 and 22 bp fragments however the mutant alleles remained intact and were not digested. Samples were sequenced to confirm the results by a DNA sequencer (ABI 3730XL system).

Results

In this study there were 126 neonates with prolonged jaundice from July 2009 to February 2011. The youngest patient was 25 days-old and the oldest, 1 year-old (123 ± 101 days). There were 52 female and 74 male patients.

In the molecular test, no patient was found to have the mutant allele; all patients were MM homozygotes (Figure 1), which was confirmed by DNA sequencing. Final diagnoses of the patients

Authors' Affiliations: ¹Transplant Research Center, Shiraz University of Medical Sciences, Shiraz, Iran, ²Department of Pathology, Shiraz University of Medical Sciences, Shiraz, Iran, ³Department of Pediatrics, Shiraz University of Medical Sciences, Shiraz, Iran.

Corresponding author and reprints: Bita Geramizadeh MD, Department of Pathology, Transplant Research Center, Shiraz University of Medical Sciences, Shiraz, Iran, P.O. Box 71345-1864. Telefax: 71345-864, E-mail: geramib@sums.ac.ir

Accepted for publication: 11 July 2012

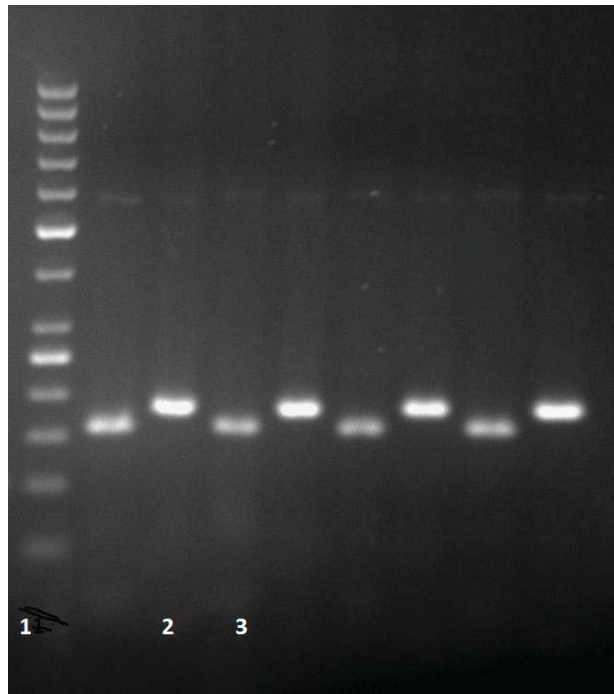


Figure 1. PCR results of patients with MM genotypes. Z Mutation: **1)** 50 bps ladder; **2)** PCR product \rightarrow 179 bp; **3)** MM genotype \rightarrow 157 bp.

were biliary atresia (50%), neonatal hepatitis (38%), and bile duct paucity (12%).

Discussion

AAT deficiency causes excess protease in the human plasma. AAT is encoded by the Pi gene located on chromosome 14q32.⁶ AAT is produced mainly by hepatocytes, where it then enters the circulation and diffuses to the lung to inhibit neutrophil elastase.¹

Variants are classified according to the Pi system. The majority of the normal population have the M allele (PiMM phenotype), which expresses a normal level of AAT in plasma.² Deficiencies in AAT that predominantly arise from the Z and S variants result in an increased risk of lung and liver disease.³ The most important variant is the Z mutation (Glu342Lys), which causes neonatal hepatitis, juvenile cirrhosis, and adult hepatocellular carcinoma.⁷

Previous studies have shown that the AAT deficiency (PiZZ and PiMZ) is one of the most common etiological factors associated with chronic liver disease in Caucasians,⁴ but not amongst Asians. A comparison between the incidences of AAT deficiency in various geographic locations of Asia and Europe shows significant differences in patients with liver disease. Reports from the Philippines,⁸ India,⁹ and Thailand¹⁰ show an incidence of zero or close to zero. The reported incidence of this genetic deficiency in European neonates with liver disease is between 5% – 15%.⁵ There are very few studies on the role of AAT in neonatal cholestasis from Iran,^{11,12} of which most have not performed molecular analyses for diagnosis confirmation. A study by Lotfi et al.¹³ has used PCR-RFLP in Iranian patients with various liver and pulmonary diseases, and the results have shown a very low incidence of homozygotes (< 5%).

According to our study in a large pediatric referral center in Southern Iran, it seems that AAT genotype determination as the

cause for prolonged neonatal jaundice is not a priority for routine practice.

References

1. Luisetti M, Seersholm N. α -1 antitrypsin deficiency 1: epidemiology of α -1 antitrypsin deficiency. *Thorax*. 2004; **59**: 164 – 169.
2. De Serres FJ, Blanco I, Fernandez-Bustillo E. Estimated numbers and prevalence of PiS and PiZ deficiency alleles of α -1 antitrypsin deficiency in Asia. *Eur Respir J*. 2008; **28**: 191 – 199.
3. Karora N, Arora S, Ahuja A, Mathur P, Maheshwari M, Das MK, et al. α -1 antitrypsin deficiency in children with chronic liver disease in North India. *Indian Pediatrics*. 2010; **47**: 1015 – 1023.
4. Kalsheker NA. α -1 antitrypsin deficiency, best clinical practice. *J Clin Pathol*. 2009; **62**: 865 – 869.
5. Bruye RD, Biervliet SV, Velde SV, Winkel MV. Clinical practice, neonatal cholestasis. *Eur J Pediatr*. 2011; **170**: 279 – 284.
6. Lee SS, Lawton JWM, Ko KH, Lam KM, Lin CK. α -1 antitrypsin phenotype by isoelectric focusing in a metropolitan southern Chinese population. *J Clin Pathol*. 2001; **54**: 798 – 800.
7. Parfrey H, Mahadeva R, Lomas DA. α -1 antitrypsin deficiency, liver disease and emphysema. *Int J Biochem Cell Biol*. 2003; **35**: 1009 – 1014.
8. Tan JJ, Cutiongco -dela Paz EM, Avila JM, Gregorio GV. Low incidence of α -1 antitrypsin deficiency among Filipinos with neonatal cholestasis. *J Paediatr Child Health*. 2006; **42**: 694 – 697.
9. Khanna R, Alam S, Sherwani R, Arora S, Arora NK. α -1 antitrypsin deficiency among Indian children with liver disorders. *Indian J Gastroenterol*. 2006; **25**: 191 – 193.
10. Changsrisawat V, Pongpaew P, Jantaradsamee P, Poovorawan Y, Vivatvakin B. α -1 antitrypsin phenotype in children with liver disease in Thailand. *Asian Pac J Allergy Immunol*. 1998; **16**: 27 – 30.
11. Fallahi GH, Farahmand F, Nemat Khorasani E. Etiologic assessment of neonatal cholestasis: a six year study in Children Medical Center, Tehran. *Tehran University Medical Journal*. 2007; **65**(4): 82 – 86.
12. Rafeey M, Golzar A, Javadzadeh A. Cholestatic syndromes of infancy. *Pak J Biol Sci*. 2008; **11**(13): 1764 – 1767.
13. Lotfi AS, Mesbah-Namin SA, Goudarzi BGH, Mirakbari AZ. Determination of alpha antitrypsin phenotypes in Iranian patients. *Iranian J Biotechnology*. 2005; **3**: 249 – 254.