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Original Article

Increased Risk of Liver Cirrhosis among Egyptian Carriers of S and/or Z Mutant Alleles of Alpha-1-Antitrypsin Gene

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Abstract

Background and Study Aims

Alpha-1-antitrypsin (A1AT) deficiency (Z and S mutations) is the most common inherited metabolic disorder with potential injury to liver and lung. The aim of this study is to determine the frequency of S and Z mutations of A1AT deficiency among Egyptian patients with liver cirrhosis and test their contribution as a risk factor to the development of the disease.

Patients and Methods

In a cross-sectional study 27 children (< 18 years) and 36 adult patients (> 18 years) suffering from liver cirrhosis in addition to 35 randomly selected, unrelated healthy control subjects were enrolled. Cases were recruited from Hepatology and Tropical Units, Mansoura University Hospitals. S and Z or M (wild) alleles were tested for all included subjects using PCR amplification of specific part of the gene followed by restriction enzyme (Taq1) digestion with analysis of RFLP (Restriction Fragment Length Polymorphism).

Results

Homozygosity for S (SS) and Z alleles (ZZ) were noted among 7.4% and 11.1% of childhood cases and 11.1% and 5.6% of adult cases compared to 8.6% and 0.0% of controls respectively. Heterozygous S (MS) was found among 25.9% of childhood cases, 30.6% of adult cases compared to 8.6% in controls. Compound heterozygotes (SZ) were found with higher frequency among controls (11.4%) than childhood cases (7.4%) and adult cases (0.0%). Gene frequency of S allele was noted higher in childhood and adult cases than controls (24.1%, 26.39%, 18.6%), while the Z allele was higher in childhood cases than adult and controls (14.8%, 6.94%, 8.6%). In addition, significant high relative risk was found among S carries and ZZ homozygotes in both studied groups.

Conclusion

Relative increased frequency of mutant A1AT deficiency alleles within cases of liver cirrhosis may be an actual risk factor taking into consideration the interaction of other genetic or environmental factors as metabolic errors, malnutrition, viral infections like HCV, schistosomiasis and diabetes. Therefore A1AT gene analysis might be an essential diagnostic and/or prognostic parameter among family members of liver cirrhosis cases.

Key Words liver cirrhosis, aetiology, alpha-1-antitrypsin, genetics, diagnosis

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INTRODUCTION

Alpha-1-antitrypsin (A1AT) is a blood protein that diffuses into tissue spaces protecting them from being harmed by enzymes released from damaged cells. A1AT deficiency is a common inherited metabolic disorder characterized by retention of the liver-produced protein A1AT in liver and low serum A1AT level. This deficiency is common in infants and children having cirrhosis, adults complaining of decompensated liver disease and might be associated with premature development of pulmonary emphysema[1-4]. A1AT inhibits a broad spectrum of serine proteases defending tissues from proteolysis; so its deficiency will result in wide varied manifestations due to the effect of leukocytes elastase on connective tissue of lungs as well as most of serine proteases including pancreatic and neutrophil elastase, neutrophil cathepsin G, pancreatic trypsin and chymotrypsin, collagenase from skin and synovia, acrosin, kallikrein, urokinase and rennin[5,6]. In newborns, elevated liver enzymes with cholestasis develops in approximately 10% of cases and persists towards the end of first year of life; 3% of the affected neonates may develop fibrosis or cirrhosis in late childhood[7]. Most adults with Pi ZZ genotypes present with signs and symptoms of chronic obstructive pulmonary disease aggravated by cigarette smoking with evidence of cirrhosis in 10-40% of the affected especially among older men[7,8].

A1AT deficiency may be one of the most common serious hereditary disorders worldwide being inherited in a co-dominance fashion because of the expression of both alleles in heterozygote states. A1AT gene is part of a gene cluster called SERPIN super-gene (SERine Proteinase INhibitor); Pi gene encoding A1AT is localized to chromosome 14q32.1. There are many inherited

variants of A1AT; PiM and its subtypes is the most common allele, PiS and PiZ are deficiency alleles which vary widely in different populations; more than 60 rare variants of A1AT have been identified. Genetic epidemiologic survey studies for carrier detection worldwide revealed a total population of 4.4 billion with at least 116 million carriers (PiMS and Pi MZ) and 3.4 million deficiency allele combinations (PiSS, PiSZ and PiZZ)[9]. Furthermore, A1AT deficiency is found in different ethnics; Z allele is seen in 1-2% of US whites, 0.48% of Africa, 0.4% central Asia, 1.51% Australia and New Zealand and S allele in 2-4% of US whites, 3.1% of Africa, 0.43% in central Asia, 3.95% in Australia and New Zealand[10].

Serum A1AT level could be quantified by immuno-precipitation which is insufficient and should be combined with phenotypic analysis. The molecular analysis for detection of A1AT mutations are not routinely available in diagnostic laboratories but instead other DNA-based methods could be used e.g. RFLP, allele-specific oligonucleotide hybridization, allele-specific amplification, direct sequencing, dual-colour detection by ligase-mediated analysis, temperature or denaturing gradient gel electrophoresis and PCR-mediated site-directed mutagenesis[11].

The aim of this work is to determine the frequency of S and Z mutations of A1AT deficiency among Egyptian patients with liver cirrhosis as well as healthy controls from the same area. This will also check their contribution as a risk factor to the development of the disease interacting with other disadvantageous genetic or environmental factors as metabolic errors, malnutrition, schistosomiasis, viral infections (HCV) and diabetes. Lastly this might be useful for both clinical evaluation of affected cases and genetic counseling in related families.

PATIENTS AND METHODS

A group of 63 cases affected with chronic liver disease ending in cirrhosis were included. Twenty-seven patients were less than 18 years (mean age 8.5 ± 4.4 years) and 36 adult patients were older than 18 years (mean age 49.4 ± 4.2 years). They were recruited from the Paediatric and Adult Hepatology and Tropical Units, Mansoura University Hospitals that give medical service to the central area of the Nile Delta region of Egypt. Cases were diagnosed as liver cirrhosis based on thorough history, clinical examination and laboratory investigations such as liver function tests (serum bilirubin, albumin, ALT, AST as well as coagulation profile) and viral hepatitis markers (HBsAg, HBeAg, and anti-HCV antibodies). Imaging studies in the form of chest X-ray, abdominal ultrasound with Doppler as well as upper endoscopy were done for functional evaluation of decompensated cases. Accordingly, pediatric cases were categorized into cryptogenic cirrhosis (12 cases), autoimmune hepatitis (10 cases) and metabolic liver cirrhosis (5 cases). On the other hand all adult cirrhosis patients had HCV infection with some cases having more than one insult (15 cases were pure HCV, 8 cases developed hepatocellular carcinoma on top, 7 cases were diabetics and 6 had schistosomiasis). Also included in this study were 35 normal healthy adult subjects with negative family history of liver disease as a control group.

An informed written consent was obtained from all patients after full explanation of the procedures. All patients underwent the following work-up for A1AT genotype characterization: a peripheral blood sample of 6 ml was taken; half left to dry for biochemical tests and the other half was put on EDTA for DNA extraction and purification.

DNA Purification was done using Generation Capture Column Kit (Gentra systems, USA) where a sample applied directly to the purification matrix contained in a spin column; cells were lysed upon contact with matrix releasing DNA which is then captured by the matrix material allowing wash of any contaminants like protein, heme and RNA leaving only DNA. Finally DNA was released from the matrix using DNA Elution solution and heat without the need for precipitation[12].

Characterization of S an Z alleles was done using the technique described by Lam et al. (1999) as follows[13]:

DNA Amplification; A mixure is prepared containing PCR water 33 µl, dNTP 3 µl, 10X buffer 5 µl, MgCl2 3 µl, PF (10 pomles/µl) 3 µl, PR (10 pomles/µl) 2 µl and DNA sample to a total volume of 50µl. 3 drops of mineral oil on top of mix and 0.25 µl of Taq polymerase were added to each sample to be ready for amplification using specific primers of each allele in two separate reactions. Samples were placed in PCR machine block for a minute at 95 Cº then amplification was run as follows: once cycle (95 Cº; 7 minutes), 2 cycles each (93 Cº; 2 minutes, 61 Cº; 5minutes and 72 Cº; 1 minute), 24 cycles each (93 Cº, 45 seconds, 61 Cº; 2 minutes and 72 Cº; 1 minute) followed by elongation cycle of 72 Cº for 16 minutes.
Primers for S allele:
PF (sense) 5'-TGAGGGGAAACTACAGCACCTCG 3'
PR (antisense) 5'-AGGTGTGGGCAGCTTCTTGGTCA 3'
Primers for Z allele:
PF (sense) 5'-ATAAGGCTGTGCTGACCATCGTC 3'
PR (antisense) 5'-TTGGGTGGGATTCACCACTTTTC 3'

Digestion of amplified DNA: Using Restriction Fragment Length Polymorphism (RFLP) technique, a mixure composed of: (Taq1 enzyme 0.5µl; 1X Buffer 1 µl; BSA 1 µl and water to a total volume of 10 µl) is left in a water bath for at least 2 hours at 65 Cº, and then the digested DNA fragments were separated on agarose gel (3%) electrophoresis stained with ethidium bromide and visualized by an ultraviolet transilluminator.

Detection of A1AT allele S (Glu 264 Val) and allele Z (Glu 342 Val) mutations: After digestion, fragments of (157+22) and (100+21) were detected for wild-M allele, 179 bp fragment for Z allele and 121 bp fragment for S allele; individuals with SS, SZ, and ZZ genotypes were retested to confirm the diagnosis. The numerous other non-deficiency alleles are not detected by this method. Figure1. presents examples of the classic wild A1AT genotype MM, as well as its heterozygote MS, double heterozygous SZ and homozygous ZZ cases being detected in one run compared to a DNA size marker.

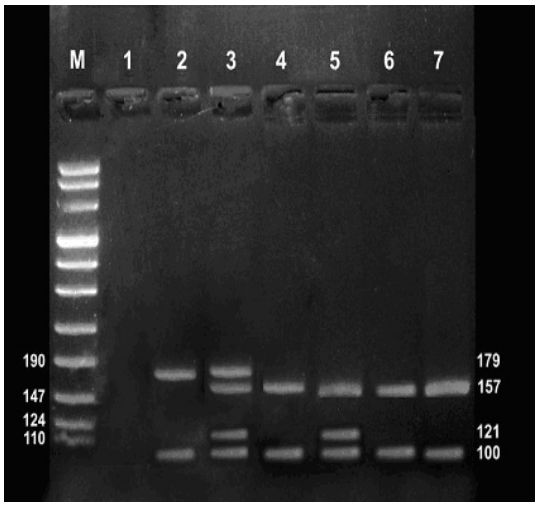

Figure 1: A1AT alleleic mutations S (121 bp) and Z (179 bp) by PCR-RFLP digested with Taq 1.
- Lane M: DNA Ladder as a marker.
- Lane 1: Water negative control.
- Lane 2: ZZ genotype (179, 100).
- Lane 3: SZ genotype (157, 121 / 179, 100).
- Lane 5: SM genotype (157, 121, 100).
- Lane 4, 6 & 7: Normal (MM) genotype (157, 100).

Statistical analysis

Allele frequency was done using gene count method (each individual is represented by 2 alleles). Genotype and allele frequency among different studied categories was assessed using Fisher's exact test, and relative risk ratio with 95% confidence interval using SPSS version 10.0.

RESULTS

Frequency of A1AT genotypes in the different studied groups is shown in Tables 1-2.

Table 1: Frequency of studied A1AT genotypes among cases of childhood liver cirrhosis compared to controls.

	Childhood Cirrhosis	Control	RR (95% CI)
Individual Genotype Frequency			
Total	27 (100%)	35 (100%)	
MM	13 (48.14%)	23(65.7%)	0.67 (0.38-1.18)
MS	7 (25.9%)*	3 (8.6%)	1.82 (1.07-3.1) #
MZ	0 (0.0)	2 (5.7%)	0 (--)
SZ	2 (7.4%)	4 (11.4%)	0.75 (0.23-2.4)
SS	2 (7.4%)	3 (8.6%)	0.91 (0.3-2.78)
ZZ	3 (10.5%)	0 (0.0%)	2.46 (1.81-3.35) #
Total Heterozygous			
MS+MZ	7 (25.9%)	5 (14.3%)	1.46 (0.81-2.62)
Total Homozygous or Compound Heterozygous			
SS+ ZZ+SZ	7 (25.9%)	7 (20.0%)	1.32 (0.73-2.37)
Individual Allele Frequency			
Total	54 (100%)	70 (100%)	
M	33 (61.1%)	51(72.9%)	0.75 (0.5-1.11)
S	13 (24.1%)	13 18.6%)	1.2 (0.76-1.87)
Z	8 (14.8%)	6 (8.6%)	1.37 (0.83-2.26)

RR (95% CI) : relative risk and 95% confidence interval
* P significant (< 0.05 by Fisher's exact test) # Significant RR

Table 2 : Frequency of studied A1AT genotypes among cases of adult liver cirrhosis compared to controls.

	Adult Cirrhosis	Control	RR (95% CI)
Individual Genotype Frequency			
Total	36 (100%)	35 (100%)	
MM	18 (51.4%)	23 (65.7%)	0.73 (0.47-1.15)
MS	11(31.4%)*	3 (8.6%)	1.79 (1.2-2.68) #
MZ	1 (2.9%)	2 (5.7%)	0.48 (0.09-2.65)
SZ	0 (0.0)	4 (11.4%)	0.0 (--)
SS	4 (11.4%)	3 (8.6%)	1.14 (0.58-2.27)
ZZ	2 (5.7%)	0 (0.0)	2.03 (1.6-2.58)#
Total Heterozygous			
MS+MZ	12 (33.3%)	5 (14.3%)	1.59(1.04-2.44) #
Total Homozygous or Compound Heterozygous			
SS+ ZZ+SZ	6 (16.7%)	7 (20.0%)	0.89 (0.47-1.69)
Individual Allele Frequency			
Total	72 (100%)	70 (100%)	
M	48 (68.5%)	51 (72.9%)	0.87 (0.62-1.21)
S	19 (27.1%)	13 (18.6%)	1.23 (0.87-1.74)
Z	5 (7.1%)	6 (8.6%)	0.89 (0.46-1.74)

RR (95% CI) : relative risk and 95% confidence interval
* P significant (< 0.05 by Fisher's exact test) # Significant RR

Alleles S and Z homozygosity among cirrhotic children was 7.4 and 11.1% versus 11.1% and 5.6% in cirrhotic adults and 8.6% and 0.0% among controls, respectively regarding heterozygosity MS genotype frequency was significantly higher in patients of all ages compared to controls (25.9%; RR=1.82 in children cases, 31.4%; OR=1.79 in adult cases versus 8.6% in controls), however MZ was absent among childhood cases. Interestingly double heterozygote SZ was absent among adult cases and in controls with a higher frequency than paediatric cases 11.4% and 7.4%, respectively). The overall gene frequency of S and Z alleles in studied chromosomes were higher for S allele among children and adult cases compared to controls; 24.1% and 27.1 versus 18.6%; however Z allele was only significantly higher in paediatric cases compared to control (14.8% versus 8.6%). Significant high relative risk was noted in MS and ZZ genotypes among both studied samples (1.82 and 2.46 respectively in paediatric group and 1.79 and 2.03, respectively in adult group).

DISCUSSION

To our knowledge few data are available on the contribution of genetically based metabolic diseases to chronic liver disease among Egyptians, thus, raising the needs for a correlative research study between these disorders in this serious national problem. In this work we selected the most common known metabolic disease alpha-1-antitrypsin deficiency (A1AT) to study the frequency of its alleles (wild normal allele M and the most common mutant deficiency alleles; S and Z).

A1AT deficiency is one of the most common serious hereditary disorders worldwide. It is transmitted in an autosomal co-dominant form and affects all major racial subgroups; African blacks, Arabs and Jews in the Middle East, Caucasians worldwide, Central Asians, Far East Asians, and Southeast Asians. This genetic disease is related to a high risk development of jaundice in infants, liver disease in children and adults and pulmonary emphysema in adults.

Moreover, carrier subjects of deficiency alleles (MS and MZ) as well as homozygous and double heterozygous individuals for these deficiency alleles (SS, SZ and ZZ) are variably susceptible to a wide variety of other adverse health effects[14] .

The prevalence of A1AT deficiency is variable among different geographic areas and ethnic groups. In Africa mean gene frequencies of S and Z alleles were 0.0638 and 0.0035 in Nigeria; 0.0362 and 0.0089 in South Africa and 0.0173 and 0.0115 in Somalia, respectively. However, in Middle Eastern countries mean gene frequencies of S and Z alleles were 0.0333 and 0.0220 in Saudi Arabia, 0.0083 and 0.0000 in Jordan and 0.0086 and 0.0000 in Tunisia, respectively[9]. In this study it was found that the S and Z allele frequencies were relatively higher among our healthy controls than the above reported African and Middle Eastern values: 0.186 and 0.086, respectively.

It is still unclear whether heterozygotes for the classic form of A1AT deficiency are predisposed to liver disease or not. Early studies of liver biopsies suggested the presence of a relationship between heterozygosity and development of liver disease[15]. This relation has been confirmed later by studies of liver biopsies taken from patients who had undergone liver transplantation; those patients showed a higher than expected prevalence of heterozygosity for the classic form of A1AT deficiency without another explanation for severe liver disease[16]. In our study, frequency of heterozygotes MS was significantly higher in all patients compared to controls, but that of heterozygotes MZ was insignificantly lower in adult patients compared to control and even absent in children cases.

It has been estimated that heterozygotes for deficient alleles had around 60% normal serum concentrations of A1AT without having any relevant clinical disease unless they have been exposed to tobacco smoke[10-17]. This interaction between genetic susceptibility and environmental factors could explain the variable clinical severity of Z allele carriers in our current study; all ZZ genotype cases had severe liver affection. Interestingly this genotype was absent among our control group, however MZ heterozygote was not reported among our childhood cases but only noticed in 2.9% of adult cases compared to 5.7% of healthy controls. Also in our study compound heterozygosity for S and Z alleles was found in 11.4% of controls and in 7.4% in childhood liver cirrhosis but absent among adult cases. It had been documented in nationwide prospective screening studies that only 10-15% of Pi ZZ population developed clinically significant liver disease over the first 20 years of life, however 85-90% had elevated serum transaminases in infancy without any obvious liver injury by age of 18 years while heterozygous patients of Pi Z allele still bear an increased risk for chronic liver disease[18,19].

A risk for liver carcinoma may be associated with A1AT heterozygotes which is related to the chronic liver disease process rather than the metabolic disorder itself[20]; however this risk is still a controversial issue. A1AT genotype frequency among patients with hepatocellular carcinoma revealed that only 5% were MS and 1.6% was MZ, however 75% of patients with bile duct carcinoma had MS and 25% had MZ genotype[21]. In our study heterozygotes for S allele (MS genotype) constituted 30.5% and 33.3% of cases diagnosed as chronic HCV and cryptogenic liver disease, respectively. However homozygous S (SS genotype) was noted in 11.11% and 20% of cases diagnosed as chronic HCV and autoimmune hepatitis, respectively. In one study, it has been demonstrated that hepatic dysfunction could develop as early as 6 month of age among heterozygote MS with subsequent development of cryptogenic cirrhosis between ages of 1 month and 18 years[22]. So, in the light of our results as well as the above reports we can speculate that S allele heterozygosity especially in association with the wild M allele is a risk factor for early onset and chronic course of liver insult which may be an important additive risk factor to HCV for future development of hepatocelluar carcinoma. Also in our study, homozygous Z (ZZ genotype) was found in about 16.7%, 20% and 5.6% of our patients diagnosed as cryptogenic liver disease, metabolic liver disease and chronic HCV, respectively. Meanwhile the overall Z allele gene frequency was found much higher in patients with chronic HCV and diabetes (33.3%) compared to other forms of chronic liver diseases.

We can conclude that there were some phenotypic-genotypic correlates between A1AT genotype and severity of liver affection. Furthermore the finding of relative increased frequency of mutant A1AT deficiency alleles within cases of liver cirrhosis together with being present in normal control subjects might constitute an actual risk factor in cases, taking into consideration other disadvantageous genetic or environmental factors as metabolic errors, malnutrition, viral infections like HCV, schistosomiasis and diabetes. Interestingly, heterozygotes MS represented a significant proportion among chronic HCV patients which may augment the future risk of malignancy in this group. Therefore A1AT gene analysis might be an essential diagnostic and even prognostic parameter among family members of liver cirrhosis cases.

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