Cutoff Level to Detect Heterozygous Alpha 1 Antitrypsin Deficiency in Turkish Population

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Background: Alpha 1 antitrypsin (AT) deficiency is a hereditary disorder leading to the defective defence system against neutrophil elastase in lung and accumulation of insoluble heterodimer AT molecules in hepatocytes. Knowledge of the prevalence of AT deficiency in each country is important to organize the public health policy. The aim of this study is to determine the prevalence of AT deficiency in Turkish population and to define the cutoff value of AT level in serum to detect heterozygous AT deficient subjects. **Materials and Methods:** Serum samples from 1,203 healthy blood donors were used, attending the Blood Bank of Hacettepe Medical Faculty. Isoelectric focusing method for determining PIM, PIS, and PIZ alleles and rate immune nephelometry for measuring the level of AT in serum were

used. **Results:** Out of 1,203 healthy blood donors enrolled, 1,164 (96.8%) had normal variant PI MM allele, 9 (0.7%) PI MZ, 7 (0.6%) PI MS, 6 (0.5%) MF, and 17 (1.4%) PI M? (unidentified variants with existing standards). Most individuals (89.6%) with low AT level (cutoff <100 mg/dl) in serum were positive for PI MM allele. The cutoff value to investigate PI MZ was 100.5 mg/dl, which had PPV and NPV of 5.0 and 99.9%, respectively. AT deficiency is a rare hereditary disorder in asymptomatic healthy Turkish blood donors. Although the cutoff value of 100.5 mg/dl for AT level in serum was able to detect heterozygous AT deficiency in the healthy population, this finding should be conformed to case-control studies. *J. Clin. Lab. Anal.* 25:296–299, 2011. © 2011 Wiley-Liss, Inc.

Key words: alpha 1 antitrypsin deficiency; blood donor

INTRODUCTION

Alpha 1 antitrypsin (AT), a glycoprotein produced mainly by hepatocytes, is a protease inhibitor which mainly has a protective role in pulmonary and hepatic tissue (1). AT deficiency causes low level of AT in serum that leads to defective protection of lung and/or accumulation of mutant AT molecule in hepatocytes associated with inflammation of the liver (2). The clinical manifestations of AT deficiency varied from severe lung and liver diseases to asymptomatic carrier state (3). The reason(s) why some people with AT

deficiency are under risk of severe lung or liver diseases are not clear. Smoking, as an environmental factor, has already shown itself as an additional risk factor for lung diseases in AT deficient individuals (3). However,

Grant sponsor: Hacettepe Medical Faculty Research Foundation; Grant number: 04 01 101 008.

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Received 17 January 2011; Accepted 13 April 2011

DOI 10.1002/jcla.20472

Published online in Wiley Online Library (wileyonlinelibrary.com).

homozygous state for PI Z was shown as an associated factor for childhood onset liver cirrhosis, besides the exact role of heterozygous state for PI MZ for developing lung or liver diseases being still a dilemma (4,5).

The AT gene is located on the long arm of chromosome 14 and has approximately 100 allele types/variants recognized by isoelectric focusing method (6). PI M is the normal allele expressing the normal level of AT in serum (150–350 mg/dl), whereas PI S and PI Z are the main deficient alleles expressing the lower levels of AT (100–200 mg/dl and 15–50 mg/dl, respectively) (7). Generally, in order to detect AT deficiency, checking the level of AT in serum is the first step followed by isoelectric focusing in case of low AT level (<100 mg/dl) (7). However, the cutoff value is controversial. The cutoff value of 100 mg/dl is 100% negative predictive for identifying PI ZZ individuals (7), whereas it misses 36% out of subjects with PI MZ (7).

The gene mapping studies noted that Northern Europe and Iberia can be the origin of the PI Z and PI S allele, respectively (8). The gene frequency reaches 23.1 per 1,000 in South Sweden for allele Z, whereas it reaches 152 per 1,000 in Portugal for allele S (8). These high rates decline toward the east and south. Greece, the western border neighbor of Turkey, has low prevalence both for S allele (2.5–28 per 100) and Z allele (2–16.2 per 100). The gene frequencies of PI Z decline continuously from Northern European to Asia and to Africa (153 Northern Europe, 36 Southern Europe, 40 Central Asia, 56 North and Middle East Africa in 10,000 population) (9). However, the migration of African population can increase the gene frequencies, and consequently has heavy influence on European genetic (10). So, the subethnic feature of the investigated population is important.

Despite the recommendations of World Health Organization on determining the prevalence of AT in every country in 1996, the epidemiological data of Turkey has not been investigated previously. Hence, this is the first study aimed at determining the rates of AT deficiency in the Turkish population. The correlation between the serum level of AT and deficient alleles of AT is also investigated.

MATERIALS AND METHOD

Patients

After informed consents were obtained, the voluntary blood donors attending the Blood Bank Unit of Hacettepe Medical Faculty were included in the study during September 2003–December 2004. All the participants were accepted as healthy blood donor according to the declaration on their health status. All of them were older than 18 years and younger than 65.

Blood samples were drawn, serum was separated from blood by centrifugation at 4°C, and stored at –70°C until AT quantitative determination or AT phenotyping.

Quantitative Determination of AT Concentration

Serum AT level was measured by the rate immune nephelometric method, (Immage[®] Immuno Chemistry System Beckman Coulter, Fullerton, CA), as per the manufacturer's instructions.

AT Phenotyping

Phenotypes were determined by isoelectric focusing (Phastsystem[®], automated electrophoresis; GE Healthcare BioSciences AB, Uppsala, Sweden). The apparatus comprises separation and control unit for electrophoresis and automated development unit for staining and destaining, which can be programmed and operated independently. Dehydrated polyacrilamide gels (PhastGel Dry IEF) were rehydrated by floating onto the rehydration solution containing Pharmalyte 4.2–4.9. All serum samples were treated with cysteine (90 µl of serum with 10 µl cysteine solution, 0.3 mol/l, pH 7.4) (11) and incubated overnight at 4°C. Cysteine reduced 0.8 µl of serum samples were applied to rehydrated polyacrilamide IEF gels. IEF running was carried out as per the manufacturer's recommended running conditions (12). Immediately after focusing, gels were fixed and stained with Phast Gel Blue R for commassie blue staining with the automated development unit (12). When intensity of the stained protein bands were poor, SilverSNAP stain kit II (Pierce, IL) for silver staining was performed as per the manufacturer's recommendations. During each experiment, control serum specimens obtained from Dr. Diane W. Cox (University of Alberta, Edmonton, Alberta, Canada), normal PI MM or abnormal phenotypes (MS, MZ, SZ, MF, and MX) were run simultaneously for comparison (12).

AT Genotyping by Polymerase Chain Reaction

Real-time PCR assay using Light Cycler technology was used for the detection of two polymorphisms E264V (denoted as Z allele) and E342K (denoted as S allele) (13) in the human AAT1 gene. For the extraction of genomic DNA, MagNA Pure LC DNA Isolation Kit I (Roche Applied Science, Germany) was used in automated MagNA Pure nucleic acid isolation instrument (Roche Applied Science, Germany). The samples were lysed by incubation with a special buffer containing chaotropic salt and proteinase K. Magnetic glass particles were added and total nucleic acids contained in the sample were bound to their surface. Unbound substances were removed with several washing steps,

then the purified total nucleic acids were eluted with low-salt buffer.

The following primer and probe sets were used for the detection of two polymorphisms E264V and E342K by real-time PCR: PiS F: GGTGCCTATGATGAAGCGT TTAGGC; PiS R: AGGTGTGGGCAGCTTCTTGGT CA; PiS FL: TTCTTCCTGCCTGATGAGGGGAA ACTA-FL; PiS LC:640GCACCTGGAAAATGAAC p; PiZ F: TCCACGTGAGCCTTGCTCGAGGCCTG; PiZ R: TTGGGTGGGATTACCACTTTTC; AATZA: CTCCAGGCCGTGCATAAGGCTGT-FL; AATZM: 705-GACCATCGACGAGAAAGGG p. Twenty microliter of PCR mixture was used and PCR results were evaluated after the melting curve analysis was performed.

Statistical Analysis

Allele and genotype frequencies were determined based on a formula available online (14). The alleles undetermined, in this study (PI SS, PI SZ, PI ZZ), was calculated from the Hardy–Weinberg Equilibrium Theory determined above. The estimated/expected number of the individuals with AT genotypes was calculated from the data from the Turkish Statistics Institute (15). SPSS for Windows, version 15.0, was used for the data analysis. Categorical comparison between AT level in serum and AT genotypes was performed by Kruskal–Wallis Variance analysis. $P < 0.005$ was considered statistically significant. Receiver operating characteristics (ROC) curve analysis was used to determine optimal cutoff value of AT level in serum for detecting AT deficient individuals (especially for PI MZ).

RESULTS

Out of 1,203 healthy blood donors enrolled, 1,164 (%96.8) had normal variant PI MM allele, 9 (%0.7) PI MZ, 7 (%0.6) PI MS, 6 (%0.5) MF, 17 (%1.4) PI M? (unidentified variants with existing standards). The other variants, namely PI ZZ, PI SS, and PI SZ alleles,

could not be detected. The calculated allele frequencies for PI M, PI S, and PI Z were 967, 3, and 4 per 1,000 populations, respectively.

The population of Turkey was 72,561,312 in December 2009, according to the data from the Turkish Statistics Institute. Accordingly, it is estimated that there was 507,929 people having PI MZ allele (prevalence of 1 per 143) and 7,256 having PI ZZ allele (prevalence of 1 per 10,000) (Table 1). Out of the 70 million people, nearly 68 million was not under a risk for AT deficiency; therefore, it can be concluded that Turkey is a low-risk country for AT deficiency.

The mean AT level in the serum of individuals with PI MZ allele was lower than the normal variant and all other variants (PI MZ 78.8 ± 28.4 mg/dl, PI MM 134.8 ± 30.3 mg/dl, PI MS 119.7 ± 24.9 mg/dl, PI MF 209.1 ± 18.0 mg/dl, PI M? 129.2 ± 41.2 mg/dl, $P < 0.00$ between PI MZ and each of the other variants). ROC curve analysis suggested that the optimum cutoff value of AT level in serum for detecting PI MZ was 100.5 mg/dl (Table 2).

PCR was used for the confirmation of S and Z variants; PI M? phenotypes were not S or Z genotypes by this method.

DISCUSSION

AT deficiency is described as a not rare but an overlooked hereditary disease, leading to severe health

TABLE 2. AT Level in Serum (100.5 mg/dl) as a Cutoff Value to Estimate PI MZ

	Estimated value	95% confidence interval
Sensitivity	0.88	0.51–0.99
Specificity	0.87	0.86–0.89
Positive predictive value	0.5	0.02–0.10
Negative predictive value	0.99	0.993–0.999

TABLE 1. Observed and Estimated Number of Genotypes in the Turkish Population^a

Genotype	Observed number of individual	Calculated frequency of genotype (per 10,000) ^b	Estimated number of people with the genotype	Calculated prevalence of the genotype (1/x)
MM	1,164	9,350.0	67,844,827	1.1
MZ	9	72.0	522,441	139.0
MS	7	70.0	507,929	143.0
SS	0	0.8	5,805	12,500.0
SZ	0	20	14,512	5,000.0
ZZ	0	1.0	7,256	10,000.0
MF	6	6.0	348,294	208.0
Total	1,203	NA	72,561,312	NA

^aNumber of the population in Turkey used for the calculations was 72,561,312. Data from Turkish Statistics Institute (December 31, 2009).

^bData calculated from Hardy Weinberg Equilibrium Formula.

NA, not applicable.

problems (16). The estimation of the prevalence of AT deficiency in each country is an important issue for the public health. This study is the first to determine AT allele frequencies in Turkish population. The frequencies of the AT alleles, PI MS, PI MZ, PI MF, are 70, 72, 48 per 10,000, respectively.

From the data mentioned above, the frequencies of PI Z and PI S can be calculated as 4 and 3 per 1,000, as expected in Turkey. The frequency of PI S is more than 90 per 1,000 in Spain and Portugal, whereas it is lower than 20 per 1,000 in Greece and Russia. Similarly, the frequencies of PI Z are more than 15 per 1,000 in Norway and Denmark, but lower than 5 per 1,000 in Russia (8). The prevalence of PIZ in India, African Americans and Mexicans from the United States were 0.4, 2.5, and 0.08, respectively (17).

The estimation of population under risk for a certain disease is a useful data to determine the public health policies. It was reported that under risk population owing to the deficient alleles (PI SS, PI ZZ, and PI SZ) in Italy, Greece, and Russia are 78,476, 11,406, and 30,786, respectively (9). However, the estimated numbers of carriers (PI MS and PI MZ alleles) should be more important for public health policies (in Italy 4,090,082 per 57,634,327 citizens, in Greece 667,155 per 10,601,527 citizens, and in Russia 4,163,438 per 146,001,176 citizens). This study revealed that approximately 1,030,370 carrier per 72,561,312 citizens are living in Turkey. And, deficient alleles are estimated to be seen in 27,573 individuals in Turkey.

The measurement of AT in serum by routine nephelometry is recommended as the first step for screening AT deficiency in patients with pulmonary and liver diseases. However, the cutoff value to search AT deficiency in these patients is not determined/settled, yet. Steiner et al. noted that the cutoff value of 100 mg/dl for AT level in serum has 100% negative predictive value and 98.6% specificity for identifying PI ZZ variant (7). However, this cutoff value has missed 36% of individuals with PI MZ, which can also be related/associated with severe health problems (18). AT level in serum was found significantly lower in PI MZ than other variants in this study. The optimal cutoff value to detect PI MZ variant was 100.5 mg/dl. These findings have confirmed that the measurement of AT level in serum by routine nephelometry is efficient and easy, and so should be the first test for searching AT deficiency in Turkish population. Further investigation to determine AT deficiency can be avoided if the level of AT is higher than 100.5 mg/dl. On the contrary, it is important to define AT deficient allele, especially PI MZ, in the cases with a low level of AT.

In conclusion, this first investigation in Turkish population revealed a low frequency of deficient AT alleles, and so supported the recent theory of the gene spreading from Northern Europe to the east and south.

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