Phenotypes of Alpha 1 Antitrypsin in Karachi, Pakistan

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Abstract

Objective: To determine serum level of the protease inhibitor, to identify phenotypes and determine their frequencies.
Study Design: A prospective study.
Setting: PMRC Research Centre, JPMC and the Aga Khan University Hospital Karachi.
Subjects: Healthy aduIts without history ol peptic ulcer disease and a normal endoscopy.
Methodology: Quantitive measurement of serum alpha 1 AT was carried out by radial immunodiffusion. phenotyping by iso-electric focusing and confirmation of phenotypes by immuno-fixation and DNA analysis technique.
Results: Serum alpha I AT was low in 13.4% of the subjects. Ni MM phenotype predominated followed by SZ SS, MZ and ZZ. DNA diagnosis accurately resolved the phenotypes as S and Z.
Conclusion: Frequency by phenotype associated with total and intermediate deficiency is less in the population (JPMA 50: , 2000).

Introduction

Serine protease inhibitor, alpha I AT is secreted y liver cells[1]. The deficiency, a common autosomal recessive disorder is characterized by reduced serum levels[2] and aminoacid substitutions of alpha I AT due to gene variation[3]. It is associated with premature development of emphysema[4], chronic liver disease and hepatocellular carcinoma[5]. The deficiency state is caused by mutations in the alpha I AT gene[6]. Apha I AT locus is polymorphic and 75 genetic variants have been identified[7] in different populations with a variable prevalance[8,9]. The commonest variants is M consisting of at least six types. MI[Va 213]. Ml[Ala213]. M2, M3, M4 and M5[10]. The most frequent variants causing alpha l AT deficiency are Z and S, formed by two different point mutations[11,12]. Studies conducted in various population indicate that gene frequencies of variants vary for different racial groups[13]. Almost complete absence of data regarding the genetic varints of alpha l AT in our population prompted us to determine serum level of the protease inhibitor, to identify phenotypes and determine their frequencies. The present findings were compared with those reported earlier[13,19,26,27].

Subjects and Methods

Blood sample from 269 healthy adults were collected and their sera atored at -70C until analyzed. There were 173 males and 96 fermales. The age range was 18-82 years (mean:36.94+0.81). All subjects gave informed consent to participate in this study wich was approved by the ethical committee of Jinnah Jostgraduate Medical Centre, Karachi. Quantitative measurement of serum alpha l AT was carried out by single radial immunodiffusion technique[14] using M paritgen immundiffusion plates (Behring Diagnostic, Marburg, Germany). Phenotyping was performed by ultrathin layer polyacrylamide gel isoelectric focusing[15]. Further confirmation of alpha l AT phenotypes was done by immunofixation[15]. IEF is a simple technique but interpretation of the banding pattern obtained by IEF is difficult at times, hence cinfirmation of phenotyping was done by DNA analysis technique[16]. A combination of polymerase chain reaction (PCR) and restriction enzyme digestion was then applied to confirm the deficient variants.

DNA was extracted from freshly drawn blood samples by the standard method[17] and was then subjected to a non radioactive PCR assay of genomic DNA for the detection of S and Z mutations in the alpha l AT gene, followed by restriction enzymes digestion[18].

Results

Serum alpha l AT concentration by RID showed a mean value of 2.63+0.05g/1 (range: 0.52-5. 51g/1). It was observed that 13.4% of the subjects mainfested low levels. Any value less then 2.0g/1 was considered as lower then normal. These low levels appear to have no diagnostic significance in most of the cases as only a few sera exhibited abnormal patterns when subjected to IEF, wich is a procedure of choice in evaluating the various phenotypes.It was found that MM predominates followed by SZ,SS, MZ, and ZZ (Figure 1).

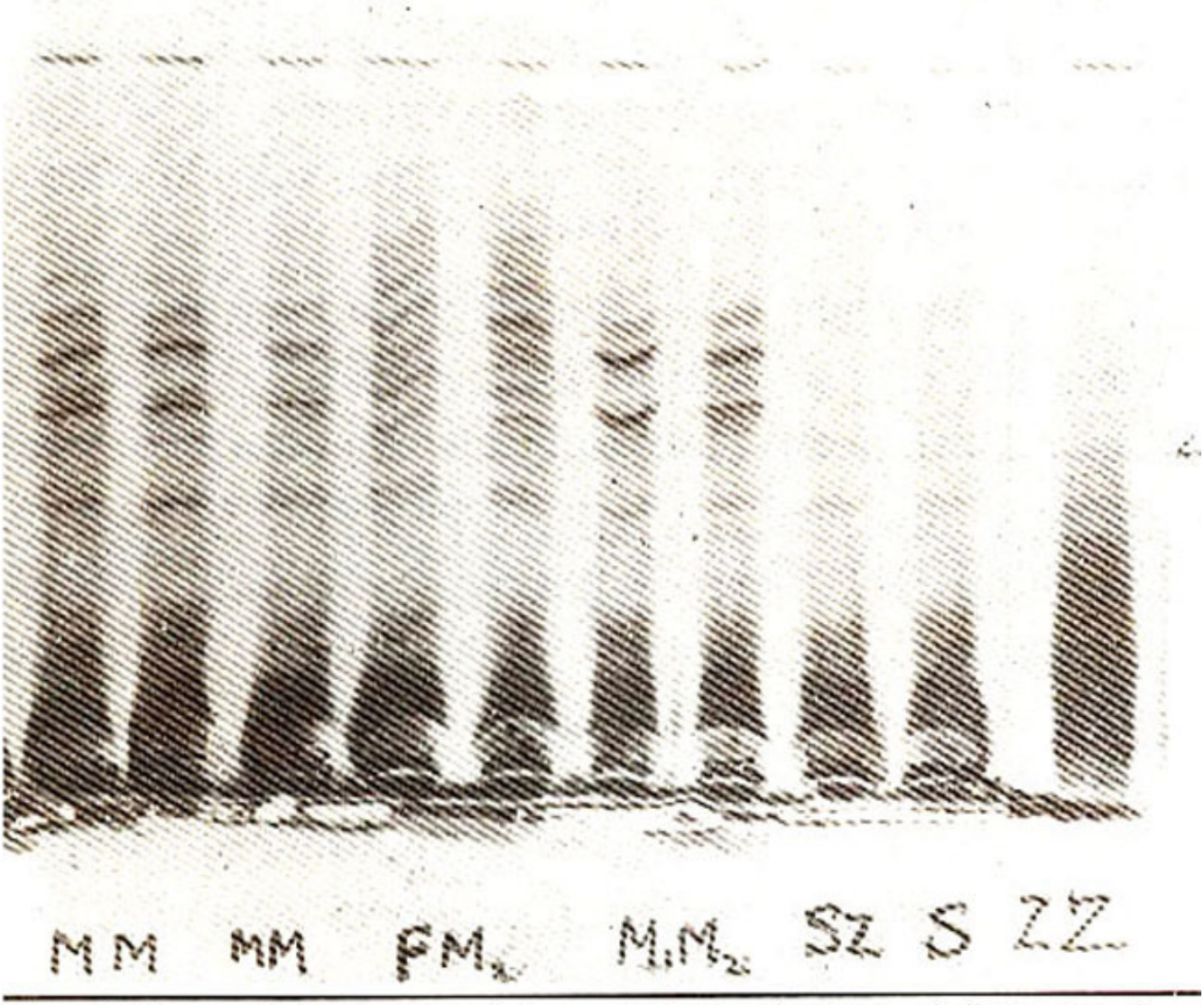

Figure 1. Variants of alpha 1 antitrypsin by isoelectric focusing. Arrows indicate point of difference in the band pattern

PiM Piz and PiS were identified in both the homozygous and heterozygous states. IEF findings were also detected by DNA based diagnostic technique, DNA diagnosis has more accurately resolved the phenotypes as S and Z. Thus genetic deficiency was confirmed by the DNA based methods which appears to be most direct and accurate way of confirming the deficient phenotypes. The results ol the typing for the S and Z mutations were in all subjects ill concordance with those of the IEF USSii\\.

Normal subjects and those h omozygous or heterozygous for the 1 or S mutations were d istingu ished unambigously (Figure:2).

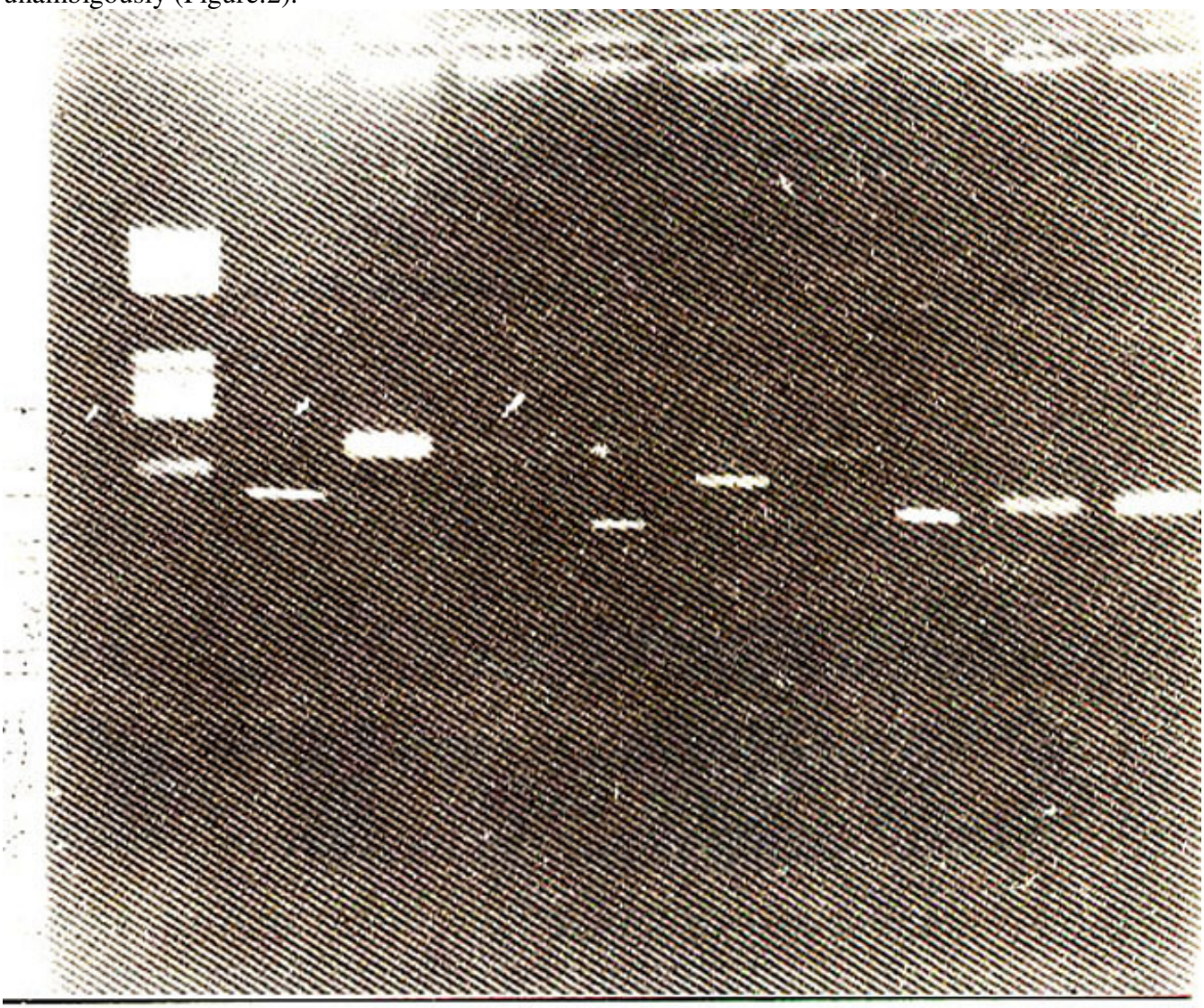

Figure 2. Agarose gel electrophoresis after digestion. Ethidium bromide stained agarose gel after electrophoresis of Asp-706 and Taq I digested and undigested products. pBR-32 Hae III (channel: 1), uncleaved PCR product (149-bp) 'S' (3,4,7), cleaved fragment (6) with mutated sequence (133-bp) and normal (111-bp) sequence (9,10). Uncleaved PCR product (97-bp) 'Z' (2), cleaved fragment with mutated (86-bp) sequence (8) and normal sequence (5)

The frequency of phenotypes is presented in Table 1.

Table 1.Frequency (%) of alpha 1 antitrypsin phenotypes in Karachi, Pakistan.

	Alpha 1 antitrypsin				
	MM	SZ	SS	MZ	ZZ
Total investigated (269)					
No. observed	263	02	02	01	01
Frequency (%)	97.7695	0.7434	0.7434	0.3717	0.3717

PIM was normal alpha I AT phenotype existing either alone or m combination with S or z variants. Liomozygous 7 variants was also identified in one subject.

Table :2 Alpha 1 Anti-Trypsin gene frequency in different populations.

Population	Number	PIM	PIS	PIZ	PIF	PII.	Reference No
Spanish	810	0.875	0.116	0.007	-	-	(24)
	644	0.878	0.116	0.005	-		
Indian	430	0.9942	-	0.0058	-	-	(27)
Italians	202	0.9501	0.0297	0.0099	0.0074	0.0025	(17)
Greeks	504	0.9600	0.0280	0.0020	0.0060	-	(26)
U.K.	4042	0.9303	0.0800	0.0141	0.0035	-	(25)
U.S.A.(Whites)	904	0.956	0.023	0.014	0.003	0.003	(18)
French	1653	0.9019	0.071	0.0142	0.0036	0.0036	(16)
Saudis	204	0.9265	0.052	0.022	-	-	(11)
Pakistani	269	0.9776	0.0074	0.0037	-	-	Present study

Table.2 illustrates the present Findings in Pakistani population with those for other populations.

Discussion

The present study indicates that MM oyerwhelm inglv predominates all other less common phenotypes described in the literature 19-22 and thus could be regarded as the normal type in Pakistan. Since our sample selection of population contained a certain number of representative from various ethnic groups living in Pakistan. it can be safely assumed that it truly represents Pakistani population in general. In another study conducted in school children where 300 samples were analyzed. MM was also found to be dominant phenotype The present findings also supports the notion that the frequency of phenotypes associated with total and those linked with intermediate deficiency of alpha I AT is substantially less in this population in comparison to European and American caucacians[23,24].

Several studies conducted in a number ol populations have shown that gene frequencies of Pi variants vary for ditlerent racial groups. The variants demonstrated in different population have a variable prevalence[8,9] The most common from is PIMM which exists in most populations at frequencies ranging from 0.8798 to 0.995825 This holds true in the study where frequency is 0.9766. The highest frequency of piS allele (0. 116) has been reported in Spanish population26 followed by 0.0800 in the British[27]. Present findings show a frequency of 0.0074 which is lower than that cited for Spanish[26]. ltal ians[20] and French[19]. The piZ variant was encountered at a gene frequency of 0.0037 which was higher than Greek[2] K and lower than that reported from ltalians[20], French[19] and Saudis[13]. The present findings show that alpha I AT polymorphism exist in Pakistani population as well and that geographical variations plays a role in the existence of various alpha 1 AT phenotypes.

Refrence

1. Stockley RA. Alpha I antitrypsin and the pathogenesis of emphysema. Lung, 1987: 165:61-77.
2. Vennarecci G. Gunson BK. Isinail T. et al. Transplantation for end liver disease related to alpha I antitrypsin. Transplantation, 1996:61:1488-95.
3. Miyake K. Wakashima M. Liver cirrhosis associated with alpha I antitrypsin deficiency, Nippon Rinsho 1994, 52:215-21.
4. Gadek JE, Fells GA, Zimmermann RL, et al Antielastases of the human alveolar structures. Implications for the protease antiprotease theory of emphysema. J. Clin. Invest., 1981, 68:889-98.
5. Eriksson S, Carlson J, Velez R. Risk of eirrhosis and primary liver cancer in alpha I AT deficiency. N. Engl, J: Med., 1986; 314:736-39.
6. Schroeder WT, Miller MF, Woo SL, et al. Charomosomal location of the human alpha I AT gene to 14q 31-32. Am. J. Hum. Genet., 1985; 37:868-72.
7. Crystal RG, Brantly ML, hubbard RC, et al. The alpha T antitrypsin gene and its mutations. Clinical consequences and straategies for therapy Chest, 1989, 95:196-208.
8. Cox DW, Smyth S. Billingsly G. Three new rare variants of alpha I antitrysin. Hum Genet., 1982;61:123-26.
9. Cox DW, Johnson AM, Fagerhol MK. Report on nomenclature meeting of alpha I antitrypsin. Hum, Genet., 1980; 53:429-33.
10. Weidinger S, Jahn W, Crijnik F, et al. Alpha lantitrypsin: evidence for a fifth PiM sub type and a new deficiency allele. PiZ Ausberg. Hum. Genet., 1985: 71:27-29.
11. Karachi K, Chandra T, Friezer DSJ, et al. Cloning and sequencing of cDNA coding for alpha I antitrypsin. Proc Natl Acad Sei. 1981:78:6826-30.
12. Long GL, Chandra T, Woo SLC, et al. Complete sequence of the cDNA for human alpha I antitrypsin and the gene for the S variant. Biochem. 1984;23:4828-37.
13. Warsy AS, Faris MA, Sedram SH, et al. Alpha I antitrypsin phenotypes in saudi Arabia. A study in the Central Province. Ann, Saudi Med., 1991;11:159-162.
14. Mancini G, Carbonara AO, Heremans JF. Immunochemical quantification of antigens by single radial immunodiffusion technique. Immunocheminstry, 1965; 2:235-254.
15. Jeppsson JO, Franzen B. Typing of genetic variants of alpha I antityspin by isoelectric focusing. Clin. Chem ., 1982: 28:219-25.
16. Lam CWK, Pang CP, Poon PMK, Yin CH. Bharathi G. Rapid screening for alpha I antitrypsin Z and S mutations. Clin Chem 1997: 43:403-404.
17. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acid Research, 1988;16:1215.
18. Andresen BS, Knudsen I, Jensen PKA, et al. Two Novel non-radioactive polymerase chain reaction based assays of dried blood spots, genomic DNA or whole cells for fast, reliable detection of Z and S mutations in the alpha I anntitrypsin gene. Clin. Chem., 1998;38:2100-107.

19. Amaud P. Chapuis Cellier C, Vittoz P, et al. Alpha I antitrypsin phenotypes in Lyon France. Hum, Genet., 1977; 39:63-68.
20. Klasen EC, ANdrea FD. Bernini LF. Phenotypes and gene distribution of alpha I antitrypsin in North Italian population. Hum. Hered., 1978; 28:474-78.
21. Dykes DD. Miller SA, Polesky HF. Distribution of alpha lantitrypsin variants in a US white population. Hum. Hered., 1984; 34:308-10.
22. Brantly M. Nuliwa T. Crystal RG. Molecular basis of alpha I antitrypsin deficieney. Am. J. Med.,1988; 84:13-31.
23. Perlmutter DH, pierce JA. The alpha I amtitrypsin gene and emphysema. Am. J. Physiol.,1989:L:147-62.
24. Sveger T. Liver disease in alpha I antitrypsin deficiency detected by sercening 200,000 infants. N. Eng;. J. Med., 1976; 294:1316-21.
25. Pongoacw P, Schelp FP. Alpha I antitrypsin protease mhibitor Phenotypes and serum concentrations in Thailand. Hum. Genet., 1980;54:119-24.
26. Estefania FJ, Carracedo AM, de Pancorb M. Alpha I antitrypsin (Pi) Subtypes in the Spanish Basque provinces. Hum. Hered., 1987; 37:233-36.
27. Cook PJL. The geneties of alpha I antitrypsin : A family study in England and Scotland. Ann. Hum. Genet., 1975; 38:275-89.
28.Fertakis A. Tsourapas A, Dourators D, et al. Pi phenotypes in Greeks. Hum. Hered., 1974; 24:313-16.