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 adults without history of 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 immunofixation and DNA analysis technique.

Results: Serum alpha 1 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 1 AT is secreted by liver cells\(^1\). The deficiency, a common autosomal recessive disorder is characterized by reduced serum levels\(^2\) and aminoacid substitutions of alpha 1 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 1 AT gene\(^6\). Alpha 1 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. M1[Val213], M1[Ala213], M2, M3, M4 and M5\(^10\). The most frequent variants causing alpha 1 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 variants of alpha 1 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 females. The age range was 18-82 years (mean:36.94±0.81). All subjects gave informed consent to participate in this study which was approved by the ethical committee of Jinnah Postgraduate Medical Centre, Karachi. Quantitative measurement of serum alpha 1 AT was carried out by single radial immunodiffusion technique\(^14\) using M partigen immunodiffusion plates (Behring Diagnostic, Marburg, Germany). Phenotyping was performed by ultrathin layer polyacrylamide gel isoelectric focusing\(^15\). Further confirmation of alpha 1 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 confirmation 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 and was then subjected to a non radioactive PCR assay of genomic DNA for the detection of S and Z mutations in the alpha 1 AT gene, followed by restriction enzymes digestion.

**Results**

Serum alpha 1 AT concentration by RID showed a mean value of 2.63±0.05g/l (range: 0.52-5.51g/l). It was observed that 13.4% of the subjects manifested low levels. Any value less then 2.0g/l 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, which 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).

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 of the typing for the S and Z mutations were in all subjects ill concordance with those of the IEF USSii\.
Normal subjects and those homozygous or heterozygous for the 1 or S mutations were distinguished unambiguously (Figure:2).

The frequency of phenotypes is presented in Table 1.
PIM was normal alpha 1 AT phenotype existing either alone or in combination with S or z variants. Liomygous 7 variants was also identified in one subject.

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

<table>
<thead>
<tr>
<th></th>
<th>MM</th>
<th>SZ</th>
<th>SS</th>
<th>MZ</th>
<th>ZZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total investigated</td>
<td>269</td>
<td>263</td>
<td>02</td>
<td>02</td>
<td>01</td>
</tr>
<tr>
<td>No. observed</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency (%)</td>
<td>97.7695</td>
<td>0.7434</td>
<td>0.7434</td>
<td>0.3717</td>
<td>0.3717</td>
</tr>
</tbody>
</table>

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

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

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

Discussion

The present study indicates that MM overwhelmingly 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 support the notion that the frequency of phenotypes associated with total and those linked with intermediate deficiency of alpha 1 AT is substantially less in this population in comparison to European and American caucacians.\textsuperscript{23,24}.
Several studies conducted in a number of populations have shown that gene frequencies of Pi variants vary for different racial groups. The variants demonstrated in different population have a variable prevalence. 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 population followed by 0.0800 in the British. Present findings show a frequency of 0.0074 which is lower than that cited for Spanish. Itilians and French. The piZ variant was encountered at a gene frequency of 0.0037 which was higher than Greek and lower than that reported from Itilians, French and Saudis. 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 I AT phenotypes.

Reference