Alpha-1-Antitrypsin Phenotypes in Rheumatoid Arthritis


Samples were collected from 131 rheumatoid arthritis patients (males and females) and 120 healthy controls. Serum $\alpha_1$-antitrypsin ($\alpha_1$AT) phenotypes were estimated by isoelectric focusing. The results showed the presence of the $\alpha_1$AT phenotypes PiM, Z and S in both heterozygous and homozygous states. The PiM subtypes: PiM1, M2, M3 and M4 were identified. The prevalence of phenotypes PiM1M1 was significantly lower in rheumatoid arthritis patients while the frequency of PiM1M2 and PiM1Z phenotypes was significantly higher ($p<0.05$).

The frequency of PiM1, PiM2, PiM3, PiM4, PiS and PiZ genes, in the patients with rheumatoid arthritis was 0.6259, 0.1946, 0.061, 0.0038, 0.0496 and 0.0649, respectively, while in the normal controls the frequency was 0.6458, 0.1750, 0.0750, 0.0208, 0.0500 and 0.033 respectively. The frequency of PiM4 was low in both groups and the difference in the frequency in rheumatoid arthritis patients and controls was statistically significant. No other statistically significant difference could be identified between the two groups.

Though the gene frequency of Pi phenotypes was not significantly different in the two groups, however, there was a positive association between $\alpha_1$AT phenotypes PiM1M2 and PiM1Z and rheumatoid arthritis.

Alpha-1-antitrypsin ($\alpha_1$AT) is a glycoprotein with the ability to combine with and inactivate several endogenous proteases particularly elastase released from neutrophils and thus plays a protective role.\(^1\text{-}^3\) It is the major protease inhibitor (Pi) in human plasma and occurs in the form of over 75 variants that can be separated by isoelectric focusing. The normal $\alpha_1$AT phenotype is PiM which exists in the form of several subtypes i.e. PiM1, PiM2, PiM3 etc., while the most common variants are PiZ, PiS and PiF.\(^4\) Several studies have reported strong associations between $\alpha_1$AT phenotypes and certain disease states.\(^1\text{-}^3,5\text{-}^7\)

Among these the most predominant association is between the phenotype PiZ and pulmonary emphysema, a destructive lung disease. The PiZ phenotype is associated with low levels of $\alpha_1$AT in the extracellular fluids of the body and hence destruction of lung tissues by elastases released from neutrophils, due to the lack of the protective mechanism provided by the $\alpha_1$AT.\(^1\text{-}^8,9\)
A few studies have reported an association between PiZ and rheumatoid arthritis,\(^{10,11}\) while others have failed to demonstrate any association.\(^{12-14}\) Due to the contradictory reports in the literature, our interest developed to investigate the frequency of \(\alpha_1\)-AT phenotypes in patients with rheumatoid arthritis. This paper presents our findings and compares them with results obtained in normal individuals.

### Patients and Methods

We studied 131 Saudi patients with definite rheumatoid arthritis as determined by the criteria of the American Rheumatism Association.\(^{15}\) The control group was made up of 120 individuals without rheumatoid arthritis. The controls were normal Saudi individuals attending outpatient clinics for minor problems. Physical examination was carried out and haematological and biochemical profiles for these patients were normal.\(^{16}\) Blood (5 ml) was collected by venepuncture in EDTA tubes and the plasma was separated from the red cells by centrifugation and stored frozen at \(-20\,^\circ\text{C}\) until required for analysis.

### Results

The observed number and the prevalence of each \(\alpha_1\)-AT phenotype identified in the rheumatoid arthritis patients and in the normal control group are presented in Table 1. The prevalence of PiM1M1 was significantly lower (\(p = 0.0034\)) and that of PiM1M2 (\(p = 0.0047\)) and PiM1Z (\(p = 0.001\)) were significantly higher in the rheumatoid arthritis patients compared with the normal controls. The overall frequency of the various phenotypes in the rheumatoid patients was significantly different from the frequency in the control group (\(\chi^2 = 29.238; \text{df} = 13; p = 0.006\)).

The total number and gene frequencies of PiM1, M2, M3, M4, S and Z genes in the rheumatoid arthritis patients and the normal control group are presented in Table 2. The frequency of PiM4 was significantly lower in rheumatoid arthritis patients compared with controls, but the number was low in both groups. The frequency of PiZ was slightly higher in the rheumatoid arthritis patients, however, the difference was not statistically significant. Similarly no difference could be identified in the frequencies of the other \(\alpha_1\)-AT variant genes in the two groups (\(p > 0.05\)).

Further grouping was carried out to study the gene frequency of PiM4 and Pi non-M, PiM1M1 and Pi non-M1M1, Pi M1 and Pi non-M1, PiS and Pi non-S, PiZ and Pi non-Z, PiS+Z and Pi non-S+Z phenotypes, in

### Table 1

Frequency of different \(\alpha_1\)-AT phenotypes in patients with rheumatoid arthritis and normal individuals

<table>
<thead>
<tr>
<th>Group</th>
<th>Total</th>
<th>M1M1</th>
<th>M2M2</th>
<th>M1M2</th>
<th>M1M3</th>
<th>M1S</th>
<th>M1Z</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rheumatoid arthritis patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Observed no.</td>
<td>131</td>
<td>44</td>
<td>6</td>
<td>34</td>
<td>12</td>
<td>12</td>
<td>17</td>
<td>6</td>
</tr>
<tr>
<td>Prevalence (%)</td>
<td>33.58</td>
<td>4.58</td>
<td>25.95</td>
<td>9.16</td>
<td>9.16</td>
<td>12.98</td>
<td>4.58</td>
<td></td>
</tr>
<tr>
<td>Normal individuals*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Observed no.</td>
<td>120</td>
<td>57</td>
<td>10</td>
<td>18</td>
<td>11</td>
<td>10</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>Prevalence (%)</td>
<td>47.5</td>
<td>8.33</td>
<td>15.0</td>
<td>9.16</td>
<td>8.33</td>
<td>1.67</td>
<td>10.0</td>
<td></td>
</tr>
</tbody>
</table>

*From Ref. 16

\(\chi^2 = 21.222; \text{df} = 6; p = 0.0016\)
Table 2
Gene frequency of α1AT phenotypes in patients with rheumatoid arthritis and normal individuals

<table>
<thead>
<tr>
<th>Pi</th>
<th>Rheumatoid arthritis</th>
<th>Normal individuals</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>χ²</td>
</tr>
<tr>
<td>M1</td>
<td>164 (0.6259)</td>
<td>155 (0.6458)</td>
<td>0.136</td>
</tr>
<tr>
<td>M2</td>
<td>51 (0.1946)</td>
<td>42 (0.1750)</td>
<td>0.203</td>
</tr>
<tr>
<td>M3</td>
<td>16 (0.0610)</td>
<td>18 (0.0750)</td>
<td>0.196</td>
</tr>
<tr>
<td>M4*</td>
<td>1 (0.0038)</td>
<td>5 (0.0208)</td>
<td>—</td>
</tr>
<tr>
<td>S</td>
<td>13 (0.0496)</td>
<td>12 (0.0500)</td>
<td>0.034</td>
</tr>
<tr>
<td>Z</td>
<td>17 (0.0649)</td>
<td>8 (0.0333)</td>
<td>2.01</td>
</tr>
<tr>
<td>Total no. of chromosomes</td>
<td>262</td>
<td>240</td>
<td>—</td>
</tr>
</tbody>
</table>

( ) = Gene frequency
*Fishers exact test.

Table 3
Comparison of α1AT gene frequencies in rheumatoid arthritis patients and normal controls

<table>
<thead>
<tr>
<th>Pi</th>
<th>Rheumatoid arthritis (gene frequency)</th>
<th>Normal individuals</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>χ²</td>
</tr>
<tr>
<td>Pi M1</td>
<td>164 (0.6259)</td>
<td>155 (0.6458)</td>
<td>0.2136</td>
</tr>
<tr>
<td>Pi non-M1</td>
<td>98 (0.3740)</td>
<td>85 (0.3542)</td>
<td>1.357</td>
</tr>
<tr>
<td>Pi M</td>
<td>232 (0.8850)</td>
<td>220 (0.9167)</td>
<td>5.041</td>
</tr>
<tr>
<td>Pi non-M</td>
<td>30 (0.1145)</td>
<td>20 (0.0833)</td>
<td>0.0004</td>
</tr>
<tr>
<td>Pi M1M1</td>
<td>44 (0.3358)</td>
<td>57 (0.4750)</td>
<td>2.635</td>
</tr>
<tr>
<td>Pi non-M1M1</td>
<td>87 (0.6641)</td>
<td>63 (0.525)</td>
<td>1.35</td>
</tr>
<tr>
<td>Pi S</td>
<td>13 (0.0496)</td>
<td>12 (0.050)</td>
<td>—</td>
</tr>
<tr>
<td>Pi non-S</td>
<td>249 (0.9504)</td>
<td>228 (0.95)</td>
<td>5.041</td>
</tr>
<tr>
<td>Pi Z</td>
<td>17 (0.0649)</td>
<td>8 (0.033)</td>
<td>2.635</td>
</tr>
<tr>
<td>Pi non-Z</td>
<td>245 (0.9351)</td>
<td>232 (0.9666)</td>
<td></td>
</tr>
<tr>
<td>Pi S + Z</td>
<td>30 (0.1145)</td>
<td>20 (0.0833)</td>
<td>1.35</td>
</tr>
<tr>
<td>Pi non-S + Z</td>
<td>232 (0.8855)</td>
<td>220 (0.9166)</td>
<td></td>
</tr>
<tr>
<td>Total no. of chromosomes</td>
<td>262</td>
<td>240</td>
<td>—</td>
</tr>
</tbody>
</table>

( ) = Gene frequency.

rheumatoid arthritis patients and controls. The results are presented in Table 3. The only significant difference was in the value of PiM1M1, which was significantly higher in the control group compared to the normal controls.

The relative risk of rheumatoid arthritis in individuals with PiM1M2 and PiM1Z phenotype was calculated and found to be respectively 1.99 and 4.32 times that in individuals without these phenotypes.

Discussion

Alpha-1-antitrypsin is an acute phase protein and its concentration is elevated in several conditions associated with trauma and inflammation.1,18 Due to the protective role played by α1AT, several pathological states are reported in individuals who inherit certain α1AT variants that are associated with decreased α1AT level or activity. The deficient variants i.e. PiZ, particularly in the homozygous state, result in significant reduction in α1AT serum level and patients with this variant often suffer from pulmonary emphysema19 and neonatal liver diseases.3 It has also been suggested that such individuals may be predisposed to erosive joint lesions caused by proteases.13

Several studies have investigated a possible association between α1AT phenotypes and rheumatoid arthritis. A few studies have reported an association with the Z heterozygotes (PiMZ and PiSZ)10,11,20 and S heterozygotes (PiMS) phenotypes12 while a number of other studies do not confirm these findings.13,21 Cox & Huber10 and Buisseret et al.11 reported an increased frequency of α1AT phenotypes which included PiZ allele in rheumatoid arthritis patients, while Sjöblom & Wollheim21 and Geddes & coworkers12 did not find any association between rheumatoid arthritis and α1AT phenotypes. However, Geddes and coworkers12 did report a significant increase in PiMZ and all non-MM phenotypes in rheumatoid arthritis with
fibrosing alveolitis. Collins & coworkers\textsuperscript{22} also reported a significant increase in non-M\textsubscript{i} variants among rheumatoid arthritis patients with obstructive pulmonary disease. More recently Michaelsh\textit{al et. al.}\textsuperscript{14} used a procedure that could separate PiM subtypes and investigated rheumatoid arthritis and systemic sclerosis patients. They showed that a highly significant association existed between the non-M\textsubscript{1}M\textsubscript{1} particularly PiM1M2\textsubscript{1}AT phenotypes and rheumatoid arthritis patients with pulmonary fibrosis. The individuals with the non-M\textsubscript{1}M\textsubscript{1} phenotype had almost a 20–30 fold increased risk of the developing pulmonary fibrosis.

Studies reported by Papiha & coworkers\textsuperscript{23} and Sanders et al.\textsuperscript{24} on a fairly large group of rheumatoid arthritis patients have shown that the increase of non-M phenotype in rheumatoid arthritis was non-significant. However, among the subvariants of M, there was a significant increase of PiM1M2 in the rheumatoid arthritis patients. The study by Papiha and coworkers\textsuperscript{25} showed that this increase in PiM1M2 was independent of an association with HLA-DR4 phenotype.

In our studies on rheumatoid arthritis patients the results showed that the prevalence of PiM1M2 and PiM1Z phenotype was significantly higher and PiM1M1 was significantly lower in the rheumatoid arthritis patients compared with the controls (p < 0.025). The gene frequencies of the Pi genes did not show any significant difference except a slightly high though non-significant PiZ and a higher PiM4 gene frequency. No difference could be seen in the frequencies of PiM and Pi non-M.

The results of this study showed a negative association between PiM1M1 phenotype and rheumatoid arthritis while PiM1M2 and PiMZ are positively associated with rheumatoid arthritis. It is difficult to present an explanation of the association of the PiM1M2 phenotypes with rheumatoid arthritis, since in vitro studies do not show any difference between the PiM subtypes in their ability to inhibit the proteases. Further functional, stability and kinetic studies are necessary to explain such an association. The association with PiMZ could be explained on the basis of reduced protease inhibitory capacity due to presence of the deficient Z allele. Various proteolytic enzymes seem to be involved in the breakdown of cartilage in various chronic arthritis, and deficient anti-protease activity could predispose to such a destruction. However, the exact mechanisms governing the positive association is not clear and further studies are required to investigate whether there is a low stability, low level, low affinity or low activity of the non-PiM1M1 phenotypes that produce an increased relative risk for the development of rheumatoid arthritis.

References


