Investigation of various Rh antigens in Eastern Province, Saudi Arabia

ABSTRACT

The frequency of RhD in Saudis is relatively high. The frequency of RhD in the Dammam region was found to be 9.18%. The gene frequency for "D" was calculated as 0.7. Rh phenotypes present in decreasing order of frequency were R^r, R^R^i, R^r^i, R^r^R^i, and R^R^R^i.

Objectives: To study the frequency of Rh antigens in the Dammam region and to compare it with other regions in Saudi Arabia.

Materials and methods:

The study was conducted on a group of 1000 individuals from the Dammam region. The blood samples were collected and Rh antigens were detected using standard serological methods.

Results:

The frequency of RhD was found to be 9.18% in the Dammam region. The gene frequency for "D" was calculated as 0.7. Rh phenotypes present in decreasing order of frequency were R^r, R^R^i, R^r^i, R^r^R^i, and R^R^R^i.

Discussion:

The findings of this study are consistent with previous studies conducted in other regions of Saudi Arabia. The high frequency of RhD in the Dammam region may be due to the genetic makeup of the population.

Conclusion:

The results of this study provide valuable information about the frequency of Rh antigens in the Dammam region, which can be used for transfusion purposes and genetic counseling.

Conflict of interest:

The authors declare no conflict of interest.
Frequency of Rh antigens ...Al Sheikh et al

Table 1 - Frequency of Rh positive/negative in Saudis in Dammam comparison with other studies in the gulf region.

<table>
<thead>
<tr>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Rh positive</td>
<td>6442</td>
<td>90.82**</td>
<td>93.8 - 96.6**</td>
<td>91.8 - 9**</td>
</tr>
<tr>
<td>Rh negative</td>
<td>651</td>
<td>9.18</td>
<td>4.3 - 6.2**</td>
<td>4.8 - 8.2**</td>
</tr>
</tbody>
</table>

*Out of those 12 were Du (0.2%)

The range represents variation between Sunni and Shia. The former having the higher frequency of Rh negatives.

Materials and methods. The study was carried out on routine blood samples of Saudis in Regional Laboratory & Blood Bank, Dammam, Eastern Province, Saudi Arabia. A total of 7709 samples were processed, (1) 7093 consecutive male Saudi donors for Rh grouping (positive/negative/Du). All Rh negative (651) samples were phenotyped for Rh antigens. (2) Another group of 416 Rh positive male Saudi donors were processed for Rh phenotyping. (3) 200 Rh positive Saudi female OPD patients were processed for Rh phenotyping.

Rh grouping was performed using conventional tube method and immediate spin. High protein monoclonal/polyclonal anti-D supplied from Dominon Biologicals was used. For Du, all negative results with anti-D were forwarded to anti-human globulin phase. Polyclonal AHG reagent was used employed from Diamed AG and results were read microscopically. For Rh phenotyping, tube method was performed using monoclonal anti-C, anti-c, anti-E and anti-e supplied from Diamed AG, Dominion Biologicals Ltd, Gamma Biologicals Inc, and Crescent Diagnostics.

Results. Frequency of Rh positive/Rh negative phenotype. Six hundred and fifty-five out of 7093 male Saudi donors were Rh negative representing 9.18%. Among 6442 Rh positives (90.82%), 12 (0.2%) were Du (see Table 1).

Frequency of Rh phenotypes. Among 616 Rh positive males and females, the distribution of various "" Rh phenotypes is shown in Table 2. Percentage were related to the total of Rh positive and negative by multiplying by 90.82 and dividing over 100. The distribution of various Rh phenotypes in Rh negative male donors is also shown in Table 2. Percentages were directly calculated out of total 7093. There were no significant differences between male donors and female patients with regard to Rh phenotypes except for slightly higher R1 and R2 and lower R0 in females (results not shown here). Among the 12 cases of Du, the phenotype in 9 cases was cDe and CcDe in remaining three.

Rh gene frequencies. Using Hardy-Weinberg equation, the frequency for 'D' was calculated as 0.7 and "d" 0.3. The frequency of common Rh genes was calculated from Table 2, so as frequency of homozygotes was obtained by multiplying by a factor of (1), while frequency of heterozygote was obtained by multiplying by a factor (0.5). Results are shown in Table 3. The gene frequency was calculated based on the most probable genotypt for given phenotype.

because, it was difficult to decide the relative contribution of R,R and R,r to cDe phenotype, a range was given.

ABO distribution among Rh positive/negative donors. Group O was commonest in both Rh positive and Rh negative representing 51.2% and 57% respectively. Group AB was particularly infrequent in Rh negative, 1% compared to 3.8% in Rh positive. Groups A and B occurred in a frequency of 24%, 18% in Rh negative donors and

Table 2 - Frequency of Rh phenotypes in Saudis in Dammam. Comparison with other nationalities.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Most common Genotype</th>
<th>Saudis Al-Sheikh 1997 %</th>
<th>Kuwaitis Al-Osir 1969 %</th>
<th>English Boorndon &amp; Dodd 1966 %</th>
</tr>
</thead>
<tbody>
<tr>
<td>CcDe</td>
<td>R1r</td>
<td>218 (32.15)</td>
<td>27.50 (34.89)</td>
<td></td>
</tr>
<tr>
<td>CDe</td>
<td>R1R1</td>
<td>143 (21.09)</td>
<td>22.00 (15.58)</td>
<td></td>
</tr>
<tr>
<td>CcDee</td>
<td>R2R2</td>
<td>54 (7.96)</td>
<td>14.50 (13.42)</td>
<td></td>
</tr>
<tr>
<td>cDe</td>
<td>R1R0</td>
<td>106 (16.53)</td>
<td>7.50 (2.06)</td>
<td></td>
</tr>
<tr>
<td>cDee</td>
<td>R2r</td>
<td>59 (8.70)</td>
<td>15.00 (11.75)</td>
<td></td>
</tr>
<tr>
<td>cDe</td>
<td>R2r</td>
<td>18 (2.65)</td>
<td>3.00 (2.33)</td>
<td></td>
</tr>
<tr>
<td>CcDe</td>
<td>R1r</td>
<td>15 (2.20)</td>
<td>2.50 (0.21)</td>
<td></td>
</tr>
<tr>
<td>CcDee</td>
<td>R2r</td>
<td>1 (0.15)</td>
<td>0.00 (0.00)</td>
<td></td>
</tr>
<tr>
<td>CDe</td>
<td>R1R1</td>
<td>2 (0.29)</td>
<td>0.00 (0.00)</td>
<td></td>
</tr>
</tbody>
</table>

Total Rh + ve* 616 (90.82) 92.00 (80.24)

cr 581 (8.19) 5.00 (15.10)
cce 58 (0.82) 1.50 (0.76)
cDe 8 (0.11) 0.00 (0.92)
cCcDe 4 (0.06) 0.50 (0.02)

Total Rh - ve* 651 (9.18) 8.00 (16.82)

*The % of Rh phenotypes for 616 Rh positive Saudis calculated out of expected 678 (taking into account expected Rh negatives also); and for 651 Rh negative donors out of 7093.
Table 3 - Calculated Rh Gene frequency in Saudis in Dammam. Comparison with other Nations.

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Genotype*</th>
<th>Saudis - Al-Sheikh 1997</th>
<th>Kuwaitis - Al-Osabi 1969</th>
<th>Whites9</th>
<th>Blacks9</th>
<th>Native Americans9</th>
<th>Orientals9</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>RHCDc</td>
<td>0.423</td>
<td>0.440</td>
<td>0.42</td>
<td>0.17</td>
<td>0.44</td>
<td>0.70</td>
</tr>
<tr>
<td>r</td>
<td>RHce</td>
<td>0.290 - 0.359§</td>
<td>0.270</td>
<td>0.37</td>
<td>0.27</td>
<td>0.11</td>
<td>0.03</td>
</tr>
<tr>
<td>R2</td>
<td>RHcDe</td>
<td>0.111</td>
<td>0.160</td>
<td>0.14</td>
<td>0.11</td>
<td>0.34</td>
<td>0.21</td>
</tr>
<tr>
<td>R0</td>
<td>RHcDe</td>
<td>0.078-0.156§</td>
<td>0.038</td>
<td>0.04</td>
<td>0.44</td>
<td>0.02</td>
<td>0.03</td>
</tr>
<tr>
<td>r1</td>
<td>RHce</td>
<td>0.004</td>
<td>0.015</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>r'</td>
<td>RHcE</td>
<td>0.0008</td>
<td>0.008</td>
<td>0.01</td>
<td>0.00</td>
<td>0.06</td>
<td>0.00</td>
</tr>
<tr>
<td>R2</td>
<td>RHcDE</td>
<td>0.015</td>
<td>0.013</td>
<td>0.00</td>
<td>0.00</td>
<td>0.05</td>
<td>0.01</td>
</tr>
<tr>
<td>r''</td>
<td>RHcE</td>
<td>0.0000</td>
<td>0.000</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

* Most probable genotype calculated from Table II.

The two Rh genes RHD and RHCE are closely linked, so crossing over is unlikely, therefore their frequency was considered together.

+ Gene Frequency calculated using formula (2 x homozygote + heterozygote) / Total number

++ Gene frequency was calculated from frequency of phenotypes.

§ For cDe phenotype both cDe/cDe and cE/cE genotypes are likely.

27%, 18% in Rh positive donors respectively.

Discussion. It was observed long time ago that Rh negative group is less common in the Gulf region and other Arab countries compared with the West. This study also supports this fact. Earlier studies in the region showed even lower frequency of Rh negative group than reported here. The difference could represent genuine difference due to continuous immigration of Saudis from different regions in the Kingdom and inter-tribal marriage.

Even though the data for the frequency of Rh negative came from male donors only, we do not expect females to have a different pattern. In fact Rh positive phenotypes were not significantly different between males donors and females patients.

Lower frequency of Rh negative in our population appears to be the reason for low risk of hemolytic disease of the newborn. The estimated risk here is 4.2% in the general population [estimated risk = % Rh negative x (% homo, Rh positive + % hetero, Rh negative x 0.5)] / 100. We found that out of 152 direct Coombs positive cord blood samples, 5.3% contained anti-D in RBC eluates. A study of 57 affected infants from a referral centre in Saudi Arabia also made similar observation and showed that disease runs moderate to severe course.

Du frequency in 7093 Saudi donors was found to be 0.2%. Hilli found 0.13% in 6809 Bahraini donors. While a survey on English donors showed incidence of 0.23%- CDc/cdE and CDc/cedcE is less common in Saudis than Whites in our study, while cDc/ee is 12 times more common in blacks than Saudis. Rh negative haplotypes and gene frequencies were found to be less compared with Western frequencies in particular r' (RHcE) and r" (RHcE). On the other hand, Rh positive haplotypes and gene frequencies were higher in particular R0 (RHcDe) and R1 (RHcDE). R2 is also more frequent in Orientals, but R0 is less frequent in them. R3 is more frequent in blacks in whom R2 is not usually high. R2 was also reported high in Kuwaitis, in whom however, R0 is not high. Theoretically, homozygotes for R-R may develop anti-c and/or anti-e, if transfused with any other Rh positive blood apart from R-R. This may suggest the need for a limited donor panel of R-R phenotype. Practically, however, anti-c and anti-e are rare in the absence of anti-D. Similarly, homozygotes for R0 may develop anti-C and/or anti-E, if transfused with any Rh positive blood apart from R0. These difficulties can be overcome, if a proper inventory of Rh phenotyped blood is maintained, so that Rh blood which is suitable in such cases is readily made available.

Gene frequencies in this study were calculated based on probabilities of the most likely genotype as is reported in the literature. We did not have access to antibodies directed against combined antigens like anti-f and anti-v. Besides direct DNA analysis for Rh genes is not yet widely available outside research labs.

In the genetic counselling of a couple, who had previously affected child with Rh hemolytic disease of the newborn, and the father has the phenotype CDe, there are higher chances of R-R genotype in the father as frequency R0 is higher in our population as compared to West. There is also higher probability of a second baby to be Rh positive. However, this probability is lower than in blacks who have much
higher frequency of R^0.
In conclusion, from our study of 7093 donors in Dammam region, we found that 90.82% were Rh positive and 9.18% were Rh negative. The Rh positive phenotypes were as follows: R'R', R'R^0', R'R^0' (or R'R), R'R, R'R^0, R'R^0, R'R^0, R'R^0 in descending order of frequency. R^0 and R^0 genes occur more frequently than reported from the West. The Rh negative phenotypes were as follows: rr, r'r', r'r and r'r in decreasing order of frequency. Future studies to delineate true genotype nature using complex antibodies and direct DNA analysis may further support our findings.

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References