ABO and Rh (D) Phenotypes, Allele Frequencies and Estimated Genotypes in Omanis: A Retrospective Study from Armed Forces Hospital

Authors
Mujtaba Allawati¹, Laila Al-Kalbani², Tufaila Al-Balushi³
¹Dept. of Medical Laboratory Science, Oman Collage of Health Sciences, Sultanate of Oman
²Dept. of Medical Laboratory, Armed Forces Hospital Al Khoudh, Sultanate of Oman

Abstract
Frequencies of ABO and Rh (D) groups vary throughout the world due to ethnic diversity. The distribution may also vary across country areas due to different ethnic groups. Oman is a genetically admixed population country consisting of African, Caucasian and Asian ancestries. Due to frequencies variation throughout the world and lack of study in Oman, this propelled us to determine the phenotypes and estimate the genetic variability of ABO and Rh (D) blood groups in the Omani population. This retrospective study concerning ABO and Rh (D) blood groups of a small section of the Omani population was undertaken between January 2017 and December 2017. The data were reviewed and calculations performed to determine ABO and Rh (D) phenotypes, allele frequencies and genotypes estimation. The results were compared with other populations.

Three thousand four hundred and sixteen (3416) blood donors' data from Armed Forces Hospital blood bank were collected. Overall distribution of ABO group in Omanis is: group O (50.47%), group A (24.06%), group B (21.63%) and group AB (3.83%), whereas distribution of Rh (D) positive and Rh (D) negative is 91.65% and 8.35% respectively. Allelic frequency of A, B, O, D and d is 0.15, 0.14, 0.71 0.71 and 0.29 respectively. Genotype frequencies recorded as (AA = 0.0225, AO = 0.213, BB = 0.0196, BO = 0.1988, AB = 0.042, OO = 0.5041, DD = 0.5057, Dd = 0.411 and dd = 0.0833). The commonest ABO blood group in Omanis is O group while the AB group is the rarest with the sequence O > A > B > AB. The recorded frequencies appear to be intermediate between some other countries and different to some other Middle East regions including Arabs.

Keywords: ABO/Rh blood group, ABO allele, ABO genes, RHD genes, allele frequency, Omani population.

Introduction
Data on ABO and Rh group distribution can be very helpful for blood bank management and safe transfusion. Knowledge on blood group frequency for different blood group antigens can be of great help on how easy is to find the matching blood units especially for recipients who require multiple transfusions. These data can also be useful in population genetics study and its association to various diseases in the population. These blood groups have also noted to be prognostic biomarkers for various cancers. Though individuals share the same blood group systems, but the frequency of specific blood type varies in the population. Prevalence of ABO and Rh groups vary throughout the world due to ethnic diversity. The distribution may also vary across country areas due to different ethnic groups. The prevalence and frequencies of
ABO and Rh groups have been largely studied in different populations including Arabs\textsuperscript{11-16}. Oman is a Gulf country with a total population of about two million\textsuperscript{17} admixed of Asian, African and Caucasian\textsuperscript{18}. A 30 years old study on 160 healthy Omanis conducted by Danubio and colleague showed the following ABO & Rh (D) allele frequencies ($A = 0.164$, $B = 0.110$, $O = 0.726$, $D = 0.738$ and $d = 0.262$)\textsuperscript{19}. However, larger sample size can be very useful for better accuracy of statistical confidence level on frequencies and distribution. Another study on 1251 Omani pregnant in Sultan Qaboos University Hospital recorded decreasing ABO types order as O (52.2%) > A (26.5%) > B (16.3%) > AB (5.1%)\textsuperscript{18}. However, this study was limited to one gender only. A recent study by Al-Riyami and colleagues on 337 Omani blood donors showed most common blood group was O+ (44.9%) followed by B+ (20.2%), A+ (17.4%), AB+ (6.8%), B- (2.7%), O- (7.4%), and A- (0.6%)\textsuperscript{20}. However, the sample size in Al-Riyami et al study gives confidence interval (C.I) of 5.36 compared to our study with 1.68 C.I. In addition, the study did not calculate the allele frequencies compared to our study. To our best knowledge, there are no other studies, which have published the ABO and Rh group and allele frequencies for Omanis. In our study, distribution of ABO and Rh (D) phenotypes and allele frequencies have been studied based on retrospective data of Armed Forces Hospital (AFH) blood bank donors.

**Methods**

A retrospective study was conducted at the Armed Forces Hospital (AFH) Al Khoudh under Ministry of Defence Oman. Ethical approval was obtained from Forces Medical Services Research Committee (FMS-RC) and Gatekeeper consent form was provided to the AFH laboratory management prior to data access. ABO and Rh (D) blood group data for 3416 male and female > 18 years old Omani donors were collected during a one-year period between January and December 2017. Ethylenediaminetetraacetic acid (EDTA) vacutainer was used for samples collection and BioRad gel card haemagglutination method was used for the phenotyping according to manufacturer's instructions. ABO and Rh Phenotypes were calculated as percentage by dividing number of specific blood type by total number of samples times 100. ABO and RHD allele frequencies were determined using Hardy-Weinberg law by the following equations, where $p$, $q$, $r$, $s$ and $t$ represent $A$, $B$, $O$, $d$ and $D$ alleles respectively:

$$p = 1 - \sqrt{(B+O)}, \quad q = 1-\sqrt{(A+O)}, \quad r = \sqrt{O}, \quad s = \sqrt{dd}, \quad t = 1 - s$$

Chi-square test of independence (Two-way Contingency Table) was performed to calculate $p$ value and determine the significance of ABO and RHD blood types distribution and frequency in Omanis compared with other populations.

**Results**

The three thousand four hundred and sixteen (3416) Omani donors consist of 3308 males and 108 females. The frequency of overall ABO types distribution pattern was found to be O (50.47%) > A (24.06%) > B (24.06%) > AB (5.1%)\textsuperscript{18}. Among ABO Rh (D) positive males and Rh (D) negative females, the distribution was noted to be the same as overall pattern, whereas it observed to be different decreasing pattern as O > B > A > AB among Rh (D) negative males and Rh (D) positive females. Among Rh (D) blood groups, 91.65% were recorded to be positive and 8.35% were negative. The decreasing order of ABO allele frequency among Omanis is $O$ (0.71) followed by allele $A$ (0.15) and allele $B$ (0.14). In Rh (D), the frequency is 0.71 for allele $D$ and 0.29 for $d$. The estimated genotype frequencies recorded as $AA = 0.0225$, $AO = 0.213$, $BB = 0.0196$, $BO = 0.1988$, $AB = 0.042$, $OO = 0.5041$, $DD = 0.5057$, $Dd = 0.411$ and $dd = 0.0833$

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### Table 1. ABO and Rh (D) types comparison between Omanis and other populations

| Study                  | O    | A    | B    | AB   | Rh (D) type (%) | Reference(s) |
|------------------------|------|------|------|------|-----------------|--------------|
| **Present**            | 50.47| 24.06| 21.63| 3.83 | 91.65          | Present study |
| Saudi Arabia           | 56.8 | 33.4 | 6    | 3.8  | 92.8           | [13]         |
| Kuwait                 | 44.6 | 26.7 | 24.1 | 4.6  | NT*            | [22]         |
| Kurdistan (Iraq)       | 37.16| 32.47| 23.84| 6.53 | 91.73          | [23]         |
| Bahrain                | 53.4 | 19.43| 23.1 | 4.07 | 92.82          | [24]         |
| Tanzania               | 52   | 26   | 19   | 3    | 98             | [12]         |
| Iran (Khuzestan)       | 40.21| 28.48| 24.71| 6.6  | 92.38          | [25]         |
| Eastern India (Jharkhand) | 34.73| 22.09| 35.15| 8.03 | 96.46          | [26]         |
| Northern India (Punjab) | 34.31| 18.01| 38.06| 9.62 | 91.28          | [27]         |
| Palestine              | 32.9 | 39.29| 21.33| 6.48 | 97.32          | [28]         |
| Jordan                 | 36.62| 38.36| 18.04| 6.98 | NT*            | [29]         |

*NT: Not tested

### Table 2. ABO and Rh (D) allele frequencies in Omani compared to other populations

| Population             | A    | B    | O    | D    | d    | Reference |
|------------------------|------|------|------|------|------|-----------|
| Oman                   | 0.15 | 0.14 | 0.71 | 0.71 | 0.29 | Present study |
| Oman historic study    | 0.164| 0.11 | 0.726| 0.738| 0.262| [19]      |
| Kuwait                 | 0.177<sup>b</sup> | 0.155<sup>a</sup> | 0.668<sup>b</sup> | NT<sup>c</sup> | NT<sup>c</sup> | [22]      |
| Saudi Arabia           | 0.197<sup>b</sup> | 0.039<sup>b</sup> | 0.764<sup>b</sup> | 0.732 | 0.268 | [13]      |
| Switzerland            | 0.29<sup>b</sup> | 0.073<sup>b</sup> | 0.637<sup>b</sup> | 0.613<sup>b</sup> | 0.387<sup>b</sup> | [30]      |
| Tanzania               | 0.159 | 0.12<sup>a</sup> | 0.721 | 0.848<sup>b</sup> | 0.152<sup>b</sup> | [12]      |
| Pakistan               | 0.183<sup>b</sup> | 0.267<sup>b</sup> | 0.55<sup>b</sup> | 0.718 | 0.282 | [32]      |
| Iraq (North Baghdad)   | 0.15 | 0.141 | 0.693 | 0.662<sup>b</sup> | 0.338<sup>b</sup> | [33]      |
| Iran                   | 0.195<sup>b</sup> | 0.172<sup>b</sup> | 0.633<sup>b</sup> | 0.724 | 0.276 | [25]      |

<sup>a</sup>P< 0.05  <sup>b</sup>P< 0.01  <sup>c</sup>Not tested
Table 3. ABO and Rh (D) genotype in Omani compared to other populations

| Genotype | Present study | Kuwait | Iraq | Jordan | Tanzania |
|----------|---------------|--------|------|--------|----------|
|          | n  | %   | n  | %   | n  | %   | n  | %   | n  | %   |
| AA       | 76.86 | 2.25  | 4  | 3.97  | 28.53 | 2.25  | 6  | 5.714 | 45.89 | 2.53 |
| AO       | 727.61 | 21.3  | 25 | 24.75 | 263.62 | 20.79 | 35 | 33.333 | 416.14 | 22.93 |
| BB       | 66.95  | 1.96  | 3  | 2.97  | 24.98 | 1.97  | 1  | 0.953  | 26.136 | 1.44 |
| BO       | 679.1  | 19.88 | 20 | 19.80 | 246.88 | 19.47 | 14 | 13.333 | 314.07 | 17.3 |
| AB       | 143.47 | 4.2   | 14 | 13.86 | 53.38 | 4.21  | 10 | 9.524  | 69.26  | 3.82 |
| OO       | 1722.01 | 50.41 | 35 | 34.65 | 650.61 | 51.31 | 39 | 37.143 | 943.504 | 51.98 |
| Total    | 1416  | 100   | 101 | 100   | 1268  | 100   | 105| 100    | 1815   | 100 |

| DD       | 1727.47 | 50.57  | NT | 555.4b | 43.8  | NTc | 1305.17b | 71.91 |
| Dd       | 1403.98 | 41.1   | NT | 566.8a | 44.7  | NTc | 467.9b   | 25.78 |
| dd       | 284.55  | 8.33   | NT | 145.8b | 11.5  | NTc | 41.9b    | 2.31 |
| Total    | 3416   | 100    | 1268 | 100   | 1815  | 100 |    |         |

*Expected ABO genotype  †Observed ABO genotype  ‡P< 0.05  §P< 0.01  ¶Not tested

Discussion

A retrospective study was conducted on 3416 Omani blood donors to determine ABO and Rh (D) phenotype, allele frequencies and estimated genotypes. The data obtained from AFH which is located in the Oman’s capital Muscat; however, blood drive campaign nature of the blood bank includes donors from different regions in Oman. Therefore, the samples were from a country wide basis and not restricted to one small area. Oman is a country of admixed ancestry of African, Asian and Caucasian. The blood group typing is very useful in genetic population\(^2\), so the study of prevalence and frequencies should be carried out in every central and regional blood bank.

The ABO and Rh (D) ratio for Omani male to female is 96.84: 3.16. However, it showed significant difference (\(P< 0.01\)) from observed ratio 94.5:5.5 on a study conducted in Pakistan by Rehman and colleagues\(^2\). Present study targeted blood donors only and not the general population, which could explain such significant difference compared to Rehman and colleague’s study. In addition, most of the samples in present study were collected from blood campaign mainly in military units where male donors were dominant. To rule out possible bias due to low number of female donors, we compared ABO and Rh (D) type of female gender (\(n = 108\)) in present study with study conducted by Al-Dughaishi and colleagues\(^1\) on larger number of Omani females (\(n = 1251\)). The statistical data showed no significant difference gender wise in ABO (\(p = 0.3190\)) and Rh (D) (\(p = 0.1483\)) types between both studies. Present study also showed no significant difference between Omani male and female blood donors with regard to ABO types (\(p = 0.9632\)) and Rh (D) types (\(p = 0.2905\)).

The overall ABO distribution pattern in Omanis showed group O is the highest, which is similar to those reported in Saudi Arabia\(^13\), Kuwait\(^22\), Kurdistan\(^23\), Bahrain\(^24\), Tanzania\(^12\), and Iran\(^25\). This ABO distribution is different from those in Eastern (Jharkhand) and Northern (Punjab) India where type B is prevalent\(^26\) and Palestine\(^28\) and Jordan\(^29\) where type A is the commonest (Table 1). Rh (D) positive and negative phenotype frequencies vary between populations. Present study recorded Rh (D)
positive frequency of 91.65% and Rh (D) negative frequency of 8.35%. This is similar to some countries in the South Eastern Arabic Peninsula (Table 3). Rh (D) negative frequency in Oman is different from Palestine, Eastern India and Tanzania where its frequency is low (Table 1) and Switzerland\textsuperscript{30} and Southwestern Ethiopia\textsuperscript{31} where its frequency is as high as 15% and 19.37% respectively.

As ABO blood group system has been widely studied and reported, we compared present study ABO allele frequencies with those from other Asian, European and African populations. Historical ABO and D allele frequencies from healthy Omani population \((n = 160)\textsuperscript{23}\) were reviewed and compared with present study. We found no significant difference between both studies \((p = 0.8377)\), which means the frequencies remained the same despite possible naturalization during the last 30 years. A significant difference between Omani population and many other reviewed populations \((p < 0.05 \text{ or } < 0.01)\) with regard to ABO alleles were observed, except with Tanzania and Iraq where similarities were recorded\textsuperscript{12,13,22,25,30,32,33} (Table 2). The background of admixed ancestry of Omani population should be considered as a reason of such difference with other populations. Political turmoil in 1964, encouraged the migration of Omanis to return to their home country from Zanzibar\textsuperscript{34} which might explain the agreement in ABO alleles \((A \text{ and } O)\) data between Oman and Tanzania. Though Omani population showed agreement with Tanzania and Iraq with regard to ABO alleles, but significant difference in the presence of D allele were recorded (Table 2).

Based on genotype frequencies, we calculated expected AA, AO, BB, BO, AB, OO, DD, Dd and dd frequencies in present study and compared it with observed or expected results of other populations (Table 3). There is no significant difference between Omani, Kuwaiti, Iraqi and Tanzanian with regard to AA, AO and BB genotypes whereas Jordanian showed statistical difference with AA \((p < 0.05)\), AO \((p < 0.01)\), AB \((p < 0.01)\) and OO \((p < 0.01)\) genotypes. Tanzanian showed agreement with Omani population except with BO \((p < 0.05)\) genotype and DD, Dd and dd \((p < 0.01)\) genotypes, whereas Iraqi showed difference only with regard to DD \((p < 0.01)\), Dd \((p < 0.05)\) and dd \((p < 0.01)\) genotypes. The significance difference in ABO and RHD genotypes reflected in the fact that allele frequencies of specific type showed significant difference with these populations.

**Conclusion**

ABO and Rh (D) frequencies are similar to the historic study on healthy Omanis reported in 1987 with a small sample size. These recorded frequencies appear to be intermediate between some other countries. The commonest blood group in Omanis is O group while the AB group is the rarest. Our study could be of great help in transfusion service, geographical information, clinical studies with disease association and genetic studies. We recommend ABO and Rh(D) study on different regions in Oman to observe any diversity. Observed ABO and Rh (D) genotyping analysis are also required to assess the agreement of estimated genotypes in present study.

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