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RESEARCH ARTICLE

FREQUENCY AND DISTRIBUTION OF A_2 AND A_2B SUBGROUPS AMONG BLOOD DONORS AT MGM KAMOTHE BLOOD BANK, NAVI MUMBAI

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ABSTRACT

Background: Group A individuals are further subdivided into A_1, A_2 and other rare types like A_3, A intermediate (int.), A_x, A_m, A_{end}, A_y, A_{el}, etc. Subgroups of A can result in discrepancy in ABO blood typing. The occurrence of weak variants due to heterogeneity of the A and B alleles poses a challenge for immunohaematology practice.

Materials and Methods: A retrospective study of 2.5 years from January 2013 to July 2015 carried out at the Department of Immunohaematology & Blood Transfusion of MGM Hospital, Kamothe, Navi Mumbai. Data has been collected from blood bank donor grouping records. All blood samples processed during period of observation were included in the study.

Results: Blood group records of 9,539 whole blood donors were analysed. It was found that out of total 9,539 donors 3,986 (41.78%) belonged to A_1 subgroup and 68 (0.71%) belonged to A_2 subgroup. 672 (7.04%) belonged to A_1B subgroup and 72 (0.76%) belonged to A_2B subgroup.

Conclusion: Identification & recording of subgroups is important. Weak subgroups of A antigen red cells may be mistyped as group O or B which might led to transfusion reactions in a few cases.

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INTRODUCTION

A century ago the first blood group system ABO was discovered. The occurrence of its weaker variants due to heterogeneity of the A and B alleles still poses an enigma for immunohematologists. The ABO locus on the long arm of chromosome 9 codes for blood group specific transferases which transfer N-acetyl D-galactosamine and/or D-galactose sugar terminally to the H antigen for the formation of A and/or B antigen respectively. Subgroups in the system are due to polymorphisms in the genes coding for the A gene which leads to diminished amounts of A antigens on red blood cells. The importance of subgrouping is that the A antigens in various subgroups may differ both quantitatively and qualitatively (Thakral *et al*., 2005). Group A individuals are further subdivided into A_1, A_2 and other rare types like A_3, A intermediate (int.), A_x, A_m, A_{bantu}, A_{end}, A_y, $A_{finland}$(fin), A_{el}, A_h (H- partially deficient, non-secretor), and A_{weak}. The 2 major subgroups A_1 and A_2 are differentiated on the basis of reactivity of A_1 cells but not A_2 cells with anti-A_1 lectin (Dolichosbiflorus) (Landsteiner and Levine, 1930). Group A red cells which react with both anti-A and Anti-A_1 are classified as A_1 which constitute approximately 80% of entire A blood group population. Group A cell which react with anti-A and not agglutinate with anti-A_1 are classified as A_2, making up of remaining 20% (Blood Group A Suptypes, 2008).

Anti-A_1 antibody appears as an atypical cold agglutinin in the sera of few A_2 or A_2B individuals who lack the corresponding antigen. Weak subgroups of A can be defined as those of group A subjects whose erythrocytes give weaker reactions or are non-reactive serologically with anti-A antisera than do those of subjects with A_2 red blood cells (Cartron *et al*., 1974). Subgroups of A can result in discrepancy in ABO blood typing. The occurrence of weak variants due to heterogeneity of the A and B alleles poses a challenge for immunohaematology practice.

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MATERIALS AND METHODS

Blood grouping records of 9,539 whole blood donors from January 2013 to July 2015 at MGM Kamothe blood bank were analysed. ABO and Rh-D grouping was carried out by conventional test-tube technique. The donor RBCs were washed thrice with 0.9% normal saline. Forward or cell grouping was done using monoclonal antisera anti-A, anti-B and anti-D (ERYSCREEN, Tulip Diagnostics; Goa, India). It was performed by taking one drop of 5% cell suspension mixed with two drops of anti-serum, centrifuged for one minute at 1000 revolution per minute (rpm). The results were then examined macroscopically and also under microscope for confirmation of agglutination. Reverse or serum grouping was done using in-house prepared pooled A cells, B cells and O cells. All the laboratory techniques were carried out according to the manufacturers' instructions. Blood groups were interpreted based on the agglutination pattern seen with forward and reverse grouping. Samples of group A and AB were further tested with anti-A_1 lectin (ERYBANK, Tulip Diagnostics; Goa, India) to classify them into A_1, A_2 and weak A subgroups. Whenever the agglutination was 4+ with anti-A antisera but negative with anti-A_1 lectin, the sample was considered as A_2 subgroup. A weak reaction with anti-A antisera on cell grouping along with a negative result with lectin was taken to signify a weak subgroup of A. Serum of A_2 and A_2B blood group individuals was tested with pooled A_1 cells at Room temperature and at 37°C for the presence of anti-A_1 antibody.

Agglutination was graded according to the American Association of Blood Banks standards: one solid agglutinate was graded as 4+, several large agglutinates as 3+, medium size agglutinates with a clear background as 2+ and small agglutinates with a turbid background as 1+; very small agglutinates with a turbid background were graded as weak reaction (Wk) and mixtures of agglutinated and un-agglutinated red blood cells as mixed field (mf) (Brecher, 2002). All the results were interpreted by trained immunohaematologists.

RESULTS

Blood group records of 9,539 whole blood donors over the past two and a half yearsfrom January 2013 to July 2015 at MGM Kamothe Blood Bank were analysed. It was found that out of total 9,539 donors 3,986 (41.78%) belonged to A_1 subgroup and 68 (0.71%) belonged to A_2 subgroup. 672 (7.04%) belonged to A_1B subgroup and 72 (0.76%) belonged to A_2B subgroup (Chart 1). Among total 9,539 blood donors, 4,054 (42.49%) had A blood group. The prevalence of subgroups within the 4,054 A blood group donors was found to be as follows: 3,986 (98.32%) belonged to A_1 subgroup while 68 (1.68%) belonged to A_2 subgroup. Similarly, 744 (7.80%) donors had AB blood group, within which the prevalence of subgroups was found to be as follows: 672 (90.32%) belonged to A_1B subgroup while 72 (9.68%) belonged to A_2B subgroup (Table 1). No other subgroups of A could be detected in the present study due to the small number of donors phenotyped for subgroups of A. Clinically significant anti-A_1 antibody was not detected in any of the A_2 or A_2B blood group donors.

Chart 1: Distribution of A subgroups among blood donors

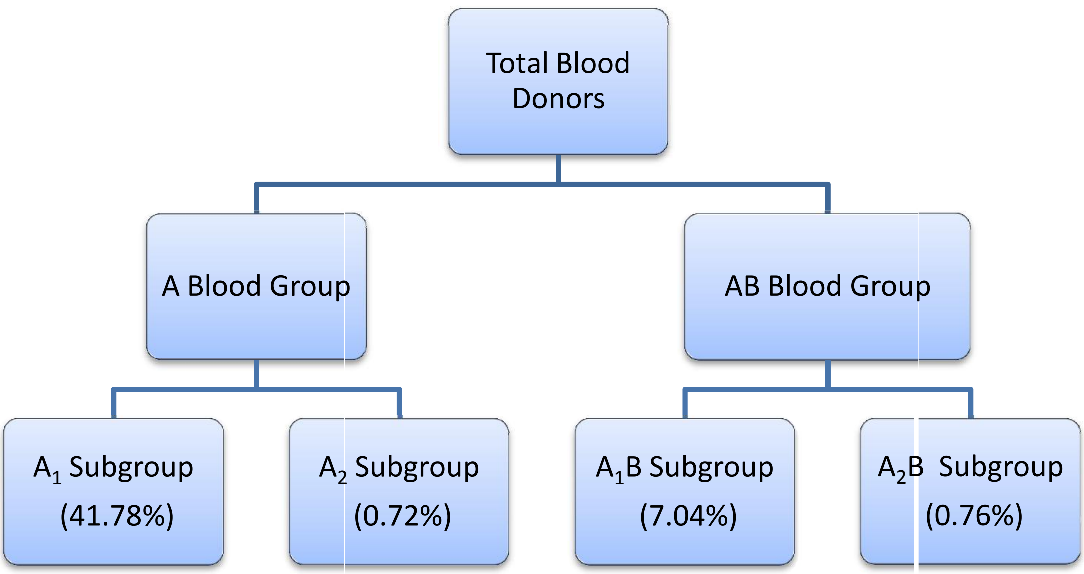

Table 1. Serological reactions of A antigen blood groups

BLOOD GROUP	Anti-A	Anti-A1	Anti-B	FREQUENCY	TOTAL
A_1	+	+	-	3,986 (98.32%)	4,054
A_2	+	-	-	68 (1.68%)	(42.49%)
A_1B	+	+	+	672 (90.32%)	744
A_2B	+	-	+	72 (9.68%)	(7.80%)

DISCUSSION

Karl Landsteiner discovered the ABO blood group system at the beginning of the 20th century and it is the most important system for clinical transfusion medicine. Epistatic addition of terminal monosaccharide immunodominant sugar to the precursor oligosaccharide H chain leads to expression of ABH antigens. Based on the red cell agglutinability and various serological reactions the blood group A can be sub-classified as A_1, A_2 and weak A sub-groups. A_1 and A_2 phenotypes account for 99% of all group A individuals. A_1 cells carry 8.1 to 11.7 x 105 antigenic sites as compared to 2.4 to 2.9 x 105 sites on A_2 RBCs thus A_1 and A_2 differ from each other both qualitatively and quantitatively (Harmening, 1999). N-acetyl galactosamine-transferase in A_2 cells less efficient at branched points than in A_1 cells due to molecular differences between the A_1 and A_2 alleles. Similar, serum glycosyltransferases show varying activity with weak positivity in A_3 and A_x to negativity in A_{end} and A_{el}. Thus varying antigenic density accounts for different strengths of agglutination reaction with monoclonal anti-A typing reagents (Thakral *et al*., 2005).

In the studies conducted by S Shastry *et al*. (2010) in Karnataka the prevalence of A_2 and A_2B was found to be 1.85% and 10.50% respectively. I.S.C. Kumar *et al*. (2012) from Andhra Pradesh found 4.1% A_2 and 19.2% A_2B subgroup prevalence in the study conducted by them. In Hiroshima the proportion of A_2 among A types was found to be 0.17%, whereas the proportion of A_2B among AB types is 1.14%; for Nagasaki, the proportions are 0.08% and 2.44%, respectively (1998). These findings of our study are similar to the above said studies. In our study we found that 98.32% donors of A blood group belonged to A_1 subgroup while 1.68% donors belonged to A_2 subgroup and 90.32% donors of AB blood group belonged to A_1B subgroup, while 9.68% donors belonged to A_2B subgroup. However in studies conducted by Sharma *et al*. (2013) from the Greater Gwalior region of India and Hassan (2010) among the Sudanese population, the prevalence of A_2B was found to be similar to that observed in our study, but the prevalence of A_2 subgroup was higher in both the studies being 8% and 14.10% respectively. The number of individuals who lack A_1 antigen is more among the AB group individualsin contrast to A group individuals. This may be due to presence of a strong B gene which suppresses A_1 antigen activity (Voak *et al*., 1970), the recessive nature of A_2gene compared to A_1 gene or requirement of a single A_2 gene and a B gene to develop as A_2B blood group phenotypically and two A_2 genes or one A_2 gene and one O gene to develop as A_2 blood group.

Ogasawara *et al*. (1998) studied the genetic basis of "excess" of A_2B subgroup as compared to A_2. Polymerase chain reaction single-strand conformation polymorphism (SSCP) and nucleotide sequence analyses were used to identify alleles. A putative recombinant allele, R101, was common in those with the A_2B phenotype but uncommon in individual with the A_2 phenotype. They concluded that R101 is presumably expressed as phenotype A_1 in R101/O heterozygous individuals, but as phenotype A_2 in R101/B heterozygotes, thus giving rise to a high frequency of A_2B phenotype (Yamamoto, 2000). For correct determination of ABO blood group status ABO genotyping is a valuable complement to serology. Mutations in the ABO alleles confer differences in the specificity and activity of transferases that add low levels of A (or B) immunodominant sugars to the precursor H antigen (Denomme *et al*., 2000). The most common A_x allele has the A_1 consensus sequence with a missense mutation encoding a Phe216Ile substitution (Olsson *et al*., 2001). A_y phenotype arises due to homozygosity for a recessive regulator gene at a locus independent of that for ABO (Weiner *et al*., 1957). Mutations in exons 6 and 7 (constituting 77% of the ABO gene) have been studied for their allelism in majority of the cases [16]. Combinations of PCR and Restriction Fragment Length Polymorphism (RFLP) or PCR with allele-specific primers have mostly been used to define polymorphism. Previously 14 definable alleles were know but Olsson and colleagues (Olsson *et al*., 2001) conducted a study and identified 15 novel A and B subgroup alleles using allele-specific primers. These included 2 mutations even outside exons 6 and 7. Thus, an individual's ABO genotype can be defined by using molecular genetics without laborious family studies. It is a useful tool for resolution of typing discrepancies and is especially valuable for distinguishing acquired variant phenotypes from inherited ones (Thakral *et al*., 2005).

A_2 and A_2B individuals may be immunologically stimulated to produce specific anti-A_1 antibody that does not cross react with A_2 red cells, but reacts with A_1 red cells since they cannot recognize A_1 antigens as being part of their own red cell make up. Weaker variants like A_2, A_2B and other subgroups of A and AB may become clinically significant when they have anti-A_1antibody reacting at 37^{o}C. This might led to incorrect ABO blood typing were AB group maybe mistyped as B groupand A group as O group. Approximately 0.4% of $A_2$and 25% of A_2B individuals have anti-A_1 in the serum (Rudmann, 1995). Individuals with an A_2B phenotype are more likely than A_2 individuals to produce anti-A_1 because of the relative reduction of A antigens on A_2B cells (Hosseini-Maaf *et al*., 2003). SimilarlyA_x individuals almost always have anti-A_1antibody in their serum whereas A_3, A_{end}, and A_{el} may occasionally have this antibody. This anti-A_1 is an antibody to type 3A and 4A branched determinants which these individuals lack. Anti-A_1 antibody usually are of no clinical significance since they agglutinate cells only up to 25°C. However, anti-A_1 can occasionally react at 37°C causing extensive destruction of A_1 cells (Boorman *et al*., 1946; Chaudhary and Sonkar, 2010).

Conclusion

Identification and recording of subgroups is important. Weak subgroups of A antigen red cells may be mistyped as group O or B. Weaker A subgroup unit which has been wrongly grouped as O, when transfused to O group individuals, the donor RBC's can show decreased survival due to the naturally occurring anti-A and anti-B antibodies present in the serum of O group individuals. Similarly AB blood group with a weaker subgroup of A may be mistyped as B. Transfusion of this mistyped AB blood to B group individual would led to a transfusion reaction due to the naturally occurring anti-A present in B group individuals. A_x individuals almost always have anti-A_1 antibodies in their serum which can be clinically significant in some cases. If their whole blood or plasma is transfused to group A individuals they can lead to fatal transfusion reactions. Hence, testing for anti-A_1 in all individuals with A subgroups should be done before transfusion, even though the presence of clinically significant anti-A_1 is rare. Thus, all A and AB blood groups should be properly evaluated for their subgroups and correct documentation of the same should be done.

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