

Imbalance in A₂ and A₂B phenotype frequency of ABO group in South India

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Background. The heterogeneity of A and B alleles results in weak variants of these antigens. Subgroups of A differ from each other quantitatively and qualitatively. The expected frequencies of A₁ and A₂ subtypes will be in Hardy-Weinberg equilibrium for the Mendelian inheritance of the allelic A₁ and A₂ genes. The frequency of A subgroups in the population from south India is not known. The aim of our study was to study the frequency of A subtypes and the prevalence of anti-A₁ antibody among this population.

Methods. Over a period of 3 years, patients' blood group was typed using a standard tube technique. Anti-A₁ lectin studies were done for all patients with groups A and AB. Based on serological reactivity the samples were classified into A₁/A₁B, A₂/A₂B and weak A subgroups. The prevalence of A subgroups was determined. The significance of differences in proportions was analysed using the chi-square test.

Results. A total of 40,113 patients' samples were typed for ABO, Rh group and A subgroups in our blood bank attached to a tertiary care hospital. Among 10,325 group A samples, 98.14% classified as A₁, 1.07% as A₂, and 0.01% as weak A; the remaining group A samples were from neonates and reacted poorly with anti A₁-lectin. The majority of AB samples (n=2,667) were of A₁B type (89.28%). However, the proportion of A₂B (8.99%) among AB samples was significantly higher than that of A₂ in group A samples (p < 0.0001). The prevalence of anti-A₁ antibodies among A₂ and A₂B samples was 1.8% and 3.75%, respectively, and none of them showed reactivity at 37°C.

Conclusion. The results of our study show a significantly higher proportion of A₂B subtypes than A₂ subgroups. A similar imbalance is seen in blacks and Japanese. The incidence of anti-A₁ antibodies is also higher among A₂B patients.

Key Words: subgroups, anti-A₁ lectin, A₂, A₂B.

Introduction

Polymorphisms in the genes coding for the ABO blood group system may lead to diminished amounts of A or B antigens on red blood cells giving rise to subgroups in the system. The occurrence of weak variants due to heterogeneity of the A and B alleles poses a challenge for immunohaematology practice. A₁ and A₂ are the major subgroups of blood group A and they differ from each other both qualitatively and

quantitatively. Subgroups of A can result in discrepancy in ABO blood typing. Red cells from people with A₁ and A₂ subgroups both react strongly with monoclonal anti-A reagents in direct agglutination tests. The distinction between these two subgroups is, therefore, made depending on the cells' reactivity with the lectin from *Dolichos biflorus* seeds. The *D. biflorus* lectin reacts specifically with cells of the A₁ subgroup, and will thus agglutinate A₁ but not

A₂ red cells. Anti-A₁ antibody appears as an atypical cold agglutinin in the sera of A₂ or A₂B 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 A₂ red blood cells¹. In the majority of cases, subgroups of A result from the expression of an alternate weak allele present at the ABO loci². The prevalence of A subgroups varies from place to place and with race. The observed frequencies of A₁ and A₂ subtypes are generally compatible with the Hardy-Weinberg equilibrium for the Mendelian inheritance of the allelic A₁ and A₂ genes. However, in some populations, such as blacks and the Japanese, the frequency of the A₂B phenotype is significantly higher than the expected frequency based on the frequency of the A₂ phenotype^{3,4}.

The prevalence of A subgroups in South India is not known. We, therefore, determined A subgroups in a large number of patients from this region. We report on the prevalence of A₂ and A₂B groups and the anti-A₁ antibody. During the study we noted that the red cells from neonates react poorly with anti-A₁ lectin.

Material and methods

Over a period of 3 years (2005-2007) blood samples from 40,113 patients were typed in the immunohaematology section of our blood bank. Blood grouping was done using the test tube technique. Forward or cell grouping was done using monoclonal antisera anti-A, anti-B, anti-AB and anti-D (Tulip Diagnostics; Goa, India) and 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 with forward and reverse grouping. In the presence of A or B antigens agglutination was observed with the corresponding antisera. The presence of circulating anti-A or anti-B antibodies was detected by reverse typing using pooled cells.

Agglutination was graded according to the AABB: 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 unagglutinated red blood cells as mixed field (mf)⁵. All the results were interpreted by a trained technologist. Samples of group A and AB were further tested with anti-A₁ lectin (Tulip Diagnostics; Goa, India) to classify them into A₁, A₂ and weak A subgroups. Whenever the agglutination was 4+ with anti-A antisera but negative with anti-A₁ lectin, the sample was considered as A₂ 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. The age of the patients was noted from the request forms. A or AB group samples which showed agglutination with pooled A cells were tested with A₁ cells to confirm the presence of anti-A₁ antibodies. The thermal amplitude of anti-A₁ was determined in each case with anti-A₁ antibodies by keeping test-tubes at 4°C, 22°C and 37°C.

Data were analysed using Graph pad - InStat version 3.10. Descriptive statistics were used to estimate frequencies. The significance of differences in proportions was analysed using the chi-square test. The overall evaluation of ABO blood group and the gene frequency estimation were performed according to the Hardy-Weinberg law. The study was conducted in compliance with the guidelines of the ethics committee of the institute.

Results

Of the 40,113 blood samples, 12,992 showed agglutination with anti-A antisera. A 4+ reaction with anti-A but no agglutination with anti-A₁ lectin was observed in 1.85% of group A and 10.50% of group AB samples. A total of seven samples gave a weak reaction (2+/mf) with anti-A and no reaction with anti-A₁ lectin (Table I).

Among 479 samples which reacted poorly with anti-A₁ lectin, 125 were from newborn babies [81/192 (A₂), 40/280 (A₂B), 4/6 (A weak subgroup, B)].

Anti-A₁ antibodies were found in two patients with A₂ group (1.8%), one with weak A subgroup, nine with A₂B group (3.75%) and two with A (weak subgroup) B. The thermal amplitude of anti-A₁ antibodies in all cases was from 4°C to room temperature and none of the antibodies reacted at 37°C.

The overall evaluation of the ABO group system

Table I - Serological reactions of suspected cases of weak A subgroups

No. of samples	Anti-A	Anti-B	Anti-AB	Anti-D	A ₁ cells	A ₂ cells	O cells	Anti-A ₁ lectin	Anti-H lectin	Interpretation
1	2+/mf	0	Wk	4+	1+	0	0	0	3+	Weak A subgroup
4										
New born	2+	4+	3+	4+	0	0	0	0	2+	AB
2	2+/mf	4+	3+	4+	1+	0	0	0	3+	A (weak subgroup) B

showed that 39.76% (n=15,947) of the samples were of blood group O, 27.86% (n=11,174) of blood group B, 25.74% of group A and 6.64% of group AB. Gene frequencies p, q and r representing the A, B and O genes were 0.1787, 0.1917 and 0.6306, respectively. The proportion of distribution is in accordance with the Hardy-Weinberg law.

Discussion

The blood group A can be sub-classified as A₁, A₂ and weak A subgroups (A_x, A₃, A_{end}, etc.) based on red cell agglutinability and various serological reactions. As shown in the Figure 1, the prevalence of A₂ and A₂B groups in our study was 1.85% and 10.50%, respectively. It may be noted that 81 samples out of 192 A₂ group and 40 among 280 A₂B group patients were from newborn babies. Because ABO antigens are not fully developed at birth, the red cells of neonates who are genetically group A₁ may not react or react only weakly with anti-A₁ lectin⁶. A₁ adults have approximately 0.8 x 10⁶ antigen sites per red cell. A₂ adults have approximately 0.24 x 10⁶ antigen sites per cell, which is comparable to the

number of antigen sites per cell seen in A₁ neonates (0.25-0.37 x 10⁶). Even though the serological results mimic those of the A₂ group, it may be wise not to categorise subgroups during the neonatal period. If these reactions are considered as false negative, the true prevalence of A₂ and A₂B in our study becomes 1.075% and 8.99%, respectively.

Our study findings in the South Indian population are similar to those in blacks and Japanese, in whom the prevalence of A₂B is significantly higher than the A₂ subgroups (p<0.0001)^{3,4}. To explain the high frequency of A₂B in black populations, the presence of a strong B gene that would suppress A₁ antigen activity has been postulated⁷. In Hiroshima the proportion of A₂ among A types is 0.17%, whereas the proportion of A₂B among AB types is 1.14%; for Nagasaki, the proportions are 0.08% and 2.44%, respectively. The excess of serological blood type A₂B in these populations is attributed partially to the suppressed synthesis of AI substance by the coexisting B enzyme in heterozygous AB individuals. According to Yoshida *et al.*, there are other unidentified factors that also contribute to the large imbalance of

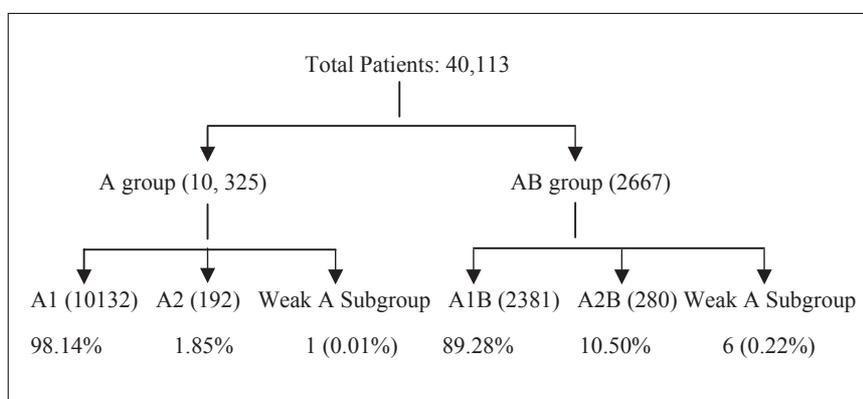


Figure 1 - Chart showing the results of initial blood grouping

serological A subtypes in the Japanese³. In order to understand the genetic basis of this "excess" of A₂B, Ogasawara *et al.* examined ABO alleles in individuals with A₂-related phenotypes. Alleles were identified by means of polymerase chain reaction single-strand conformation polymorphism (SSCP) and nucleotide sequence analyses. The frequencies of A₂-related alleles were clearly different between the A₂ and A₂B phenotypes. A putative recombinant allele, *R101*, was uncommon in individual with the A₂ phenotype, but common in those with the A₂B phenotype. They concluded that *R101* is presumably expressed as phenotype A₁ in *R101/O* heterozygous individuals, but as phenotype A₂ in *R101/B* heterozygotes, thus giving rise to a high frequency of A₂B phenotype⁴.

A₂ and A₂B individuals may have anti A₁ in their serum. Approximately 0.4% of A₂ and 25% of A₂B individuals have anti-A₁ in the serum⁸. Anti-A₁ antibody usually agglutinates cells only up to 25°C and are of no clinical significance. However, anti-A₁ can occasionally be a clinically significant antibody when it reacts at 37°C and the extensive destruction of A₁ cells has also been reported^{9,10}. We detected anti-A₁ in 1.8% of A₂ and in 3.75% of A₂B individuals. All of them were cold-reacting antibodies. As described for the *R101* allele in the Japanese population, individuals with an A₂B phenotype are more likely than A₂ individuals to produce anti-A₁ because of the relative reduction of A antigens on A₂B cells¹¹.

Like the present study, a few other studies of ABO blood group prevalence in South India have shown that group O is the most predominant one, followed by group B and group A. One study carried out at Bangalore, Karnataka showed that the frequency of group O is 39.81%, that of group B 29.95%, that of group A 23.85% and that of group AB 6.37%¹². Another study conducted on the population of the Chittur district of Andhra Pradesh showed a similar pattern of distribution of blood groups¹³.

This is the first prevalence study of A subgroups in a South Indian population. In this part of India there is a significant imbalance of A₂ in A₂B phenotypes, as in black and Japanese populations. Further studies are required on the prevalence and classification of weak A subgroups. A molecular characterization of the subtypes would have been useful in this regard, but not possible in this study. Although the presence of clinically significant anti-A₁ is rare, we suggest

testing for anti-A₁ in all patients with A subgroups before blood transfusion.

References

- 1) Cartron JP, Gerbal A, Hughes-Jones NC, et al. 'Weak A' phenotypes: relationship between red cell agglutinability and antigen site density. *Immunology* 1974; **27**: 723-7.
- 2) Heier HE, Namork E, Calkoska Z, et al. Expression of A antigens on erythrocytes of weak blood group A subgroups. *Vox Sang* 1994; **66**: 231-6.
- 3) Yoshida A, Dave V, Hamilton HB. Imbalance of blood group A subtypes and the existence of superactive B gene in Japanese in Hiroshima and Nagasaki. *Am J Hum Genet* 1988; **43**: 42228.
- 4) Ogasawara K, Yabe R, Uchikawa M, et al. Different alleles cause an imbalance in A₂ and A₂B phenotypes of the ABO blood group. *Vox Sang* 1998; **74**: 242-7.
- 5) Brecher ME. *Technical Manual*. 14th ed, American Association of Blood Banks; 2002.
- 6) Klein GH, Anstee DJ. *Mollison's Blood Transfusion in Clinical Medicine*, 11th ed. Oxford, Wiley-Blackwell 2006:114-9.
- 7) Voak D, Lodge TW, Reed JV. A possible explanation for the expression of A₂B phenotypes observed in some populations. *Vox Sang* 1970; **18**: 471-4.
- 8) Rudmann SV. *Textbook of Blood Banking and Transfusion Medicine*. 1st ed. Philadelphia, WB Saunders 1995: 73-5.
- 9) Boorman KE, Dodd BE, Loutit JF, et al. Some results of transfusion of blood to recipients with "cold agglutinin". *Br Med J* 1946; 751-4.
- 10) Chaudhary R, Sonkar A. High titer immunizing anti-A₁ in an A₂B patient resulting in hemolytic transfusion reaction. *Transfusion Bulletin*. Available at: <http://www.indmedica.com/journals.php?journalid=2&issueid=2&articleid=22&action=article>. Accessed on 03-03-2010
- 11) Hosseini-Maaf B, Hellberg A, Rodrigues MJ, et al. ABO exon and intron analysis in individuals with the A_{weak}B phenotype reveals a novel O^{1v}-A² hybrid allele that causes four missense mutations in the A transferase. *BMC Genet* 2003; **4**: 17.
- 12) Periyavan S, Sangeetha SK, Marimuthu P, et al. Distribution of ABO and Rhesus-D blood groups in and around Bangalore. *Asian J Transf Sci* 2010; **4**: 41.
- 13) Reddy KS, Sudha G. ABO and Rh(D) blood groups among the Desuri Reddis of Chittur District, Andhra Pradesh. *Anthropologist* 2009; **11**: 237-8.

Received: 12 November 2009 - Revision accepted: 4 May 2010

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