



Prevalence of A₂ and A₂B Subgroups and Anti-A₁ Antibody in Blood Donors in Jazan, Saudi Arabia

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Purpose: A₂ and A₂B are rare phenotypes of the ABO blood group system. Some individuals with A₂ and A₂B may have anti-A₁ antibodies that may be clinically significant or insignificant. The aim of this study was to determine the frequency of A₂, A₂B phenotypes and anti-A₁ antibodies in blood donors in Jazan, Saudi Arabia. This study also evaluated the reactivity potential of anti-A₁ antibodies.

Materials and Methods: Blood samples collected from 446 blood donors were typed for ABO (cell and serum grouping) and Rh D. Individuals with blood group A and AB were further subtyped by testing with anti-A₁ lectin. In addition to the serum grouping using A₁ red cells, A₂ and A₂B individuals were screened for the presence of anti-A₁ in their sera against A₁ red cells at 4°C, 22°C and 37°C to determine the thermal amplitude of the reacting anti-A₁ antibody (if present).

Results: Among A and AB, A₁ was the commonest phenotype (20.2%, n=90 out of 446) while A₁B was found to be 1.8% (n=8) among AB phenotype. A₂ and A₂B were found to be 2.2% (n=10) and 0.9% (n=4), respectively. Only one individual with A₂B blood type showed cold reactive anti-A₁ antibody, the strength of which was 32.

Conclusion: A₂ and A₂B were the rarest among ABO phenotypes in the studied population. Although rare, anti-A₁ antibody is not so uncommon. Care shall be taken during routine ABO grouping especially in cases of mix-field or weak positive reactions in A and AB phenotypes.

Keywords: ABO, anti-A₁, subgroups, blood groups, antibodies, Saudi Arabia

Introduction

The International Society for Blood Transfusion (ISBT) has recognized 346 blood group antigens. Out of these 346 antigens, 308 have been designated in 36 blood group systems while 38 are still not assigned to any blood group system.¹ Among all human blood group systems, ABO (ISBT designation 001) also known as histo-blood group system, is at the center of the stage. ABO blood group antigens are found on the surface of red cells, platelets, lymphocytes and cells in the vascular endothelium, intestine, cervix, urethra, and mammary glands. ABO antigens are also found in soluble form in secretions including saliva, tears, and milk.² This blood group is equally important in blood and/or blood component transfusion, hematopoietic stem cell and organ transplantation. This significance is due to the presence of “naturally occurring IgM anti-A, anti-B or anti-AB” antibodies produced by stimulated B cells directly without the assistance of helper T cells and thymus dependence against environmental agents in individuals they lack the corresponding antigen. Mismatch blood transfusion can cause severe clinical manifestations.

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Major ABO blood group antigens are A, B, AB and O. A₂ and A₂B are rare subtypes of ABO blood group system. Other less prevalent subtypes of A include A₃, A_x, A_{end}, A_y and A_{el}. Differences between A₁ and A₂ are quantitative as well as qualitative. Qualitative difference of A₁ and A₂ lies in their chemical structures. Individuals with A phenotype express A^a, A^b, A^c and A^d determinants while A₂ have only A^a and A^b antigenic determinants. Absence of A^c and A^d is assumed to be a cause of development of anti-A₁ in A₂ and A₂B individuals.²⁻⁴ Usually anti-A₁ exist as naturally occurring IgM with a thermal amplitude of less than 25°C. However, cases of anti-A₁ reacting at 37°C have also been reported in the literature.^{5,6} Anti-A₁ is important as it is one of the causes of ABO discrepancies, it can develop hemolytic transfusion reaction and its clinical manifestations have also been reported in hemopoietic stem cell and organ transplantation.^{7,8}

To the best of the authors' knowledge no local study has been conducted to evaluate the frequency of A₂, A₂B phenotypes and anti-A₁ in normal individuals in Saudi Arabia. The aim of this study was to determine the frequency of A₂, A₂B, and anti-A₁ in blood donors in Jazan, Saudi Arabia. Furthermore, this study also evaluated the reactivity potential of anti-A₁ antibodies.

Materials and Methods

This was a cross sectional study conducted at the Department of Medical Laboratory Technology, Faculty of Applied Medical Sciences, Jazan University, Jazan, Saudi Arabia. This study was approved by the Jazan Hospital Institutional Review Board and was conducted in accordance with the Declaration of Helsinki. Students who donated blood during the blood donation camp arranged in the Faculty of Applied Medical Sciences were requested to participate voluntarily in the study. Blood samples were collected, after informed written consent, in EDTA anticoagulated tubes and red top tubes for serology. Blood donors were male students aged 20±1.5 years. None of the blood donors had a clinical history of any hereditary blood disorders, had a major history during the previous year or were on medication at the time of blood donation.

All samples were typed for ABO (cell and serum grouping) and Rh status by tube method using commercially available antisera (Bio-Rad, DiaMed GmbH, Switzerland). Individuals with blood group A and AB were further subtyped by testing with anti-A₁ lectin (Bio-Rad, DiaMed GmbH, Switzerland) to classify them into

A₁, A₂, A₁B, and A₂B. In addition to the serum grouping using A₁ red cells, A₂ and A₂B individuals were screened for the presence of anti-A₁ in their sera against A₁ red cells at 4°C, 22°C and 37°C to determine the thermal amplitude of the reacting anti-A₁ antibody (if present). After incubation the cell-serum suspension was washed, treated with antihuman globulin (AHG) and results were interpreted. Sample positive for antibody was tested for the strength of reactivity. In a semi quantitative method serum was serially (1:1, 1:2, 1:4, 1:8, 1:16, 1:32 ...) diluted and reacted with A₁ red cells. Reaction in the last tube showing agglutination was noted as the titer of the antibody.

Statistical analysis was carried out through Statistical Package for the Social Sciences (SPSS version 25 IBM Corporation, Armonk, NY, USA). For the estimation of frequencies, descriptive statistics were used.

Results

A total of 446 normal healthy blood donors were typed for ABO and Rh blood grouping. Results of ABO and Rh typing are shown in Table 1. Among 446 individuals, a total of 100 were typed as A while 12 individuals were AB. Subtyping of A and AB showed 90 individuals (20.2%) to be A₁ while 10 (2.24%) were A₂ as shown in Table 2. Results of subtyping of AB are shown in Table 2.

During the ABO typing, one sample of A₂B individual showed mix-field reaction in serum grouping with A₁ red cells. Antibody screening of all A₂ and A₂B individuals also showed only one individual with A₂B blood positive for anti-A₁ at 4°C while weakly reactive at 22°C. No agglutination was observed in the prewarm tubes. Antibody titration of the serum of the individual with A₂B blood type having anti-A₁ antibody showed a strength of 32 at 4°C while only weak reactivity at 22°C.

Table 1 The Frequency of ABO Blood Typing of the Study Population

ABO Phenotype	Rh (D) Positive		Rh (D) Negative		Total	
	n	%	n	%	n	%
	A	96	21.52	4	0.9	100
B	100	22.42	4	0.9	104	23.32
AB	10	2.24	2	0.45	12	2.69
O	224	50.22	6	1.35	230	51.57
Total	430	96.4	16	3.6	446	100

Table 2 Distribution of A and AB Subgroups Among the Study Population

ABO Phenotypes	Subgroup	Frequency	Percentage
A	A ₁	90	20.2
	A ₂	10	2.2
AB	A ₁ B	8	1.8
	A ₂ B	4	0.9
Total		112	25.14

Discussion

Among ABO blood group antigens, A₂ and A₂B are rare phenotypes. These two phenotypes are differentiated from A₁ and A₁B on the basis of positive reaction with anti-A₁ lectin. In this study, 446 samples were tested for ABO typing and subtyping. Group O was the most common phenotype (51.57%). Phenotypes A, B and AB were 22.4%, 23.3% and 2.7% respectively. The majority of the subjects (96.4%) of this study were Rh D positive and the remaining 3.6% were Rh D negative. Among A and AB, A₁ (20.2%) was the commonest phenotype while A₁B was found to be 1.8% among AB phenotype. A₂ and A₂B were found to be 2.24% and 0.9% respectively. A₁, AB and O phenotyping results are similar to the locally conducted studies^{9–14} except one;¹⁵ that reports the highest frequency of O (62%), and lower frequency of B (8.7%) as compared to the current study and all other studies.

It should be noted that to date none of the studies conducted in Saudi Arabia has reported the prevalence of A₂ and A₂B. This was the first study that reported the frequencies of A₂ and A₂B. The results of the current study shows that A₂ and A₂B are rare phenotypes.

Among A₂ and A₂B individuals, only one donor showed mix-field reaction in serum grouping raising the suspicion of IgM anti-A₁ in the study population. This observation was strengthened by the presence of agglutination (2+) in the test tubes incubated at 4°C, while only weak reactivity at 22°C. The antibody was demonstrable after incubation at 4°C and 22°C indicating its IgM nature. None of the A₂ individuals was positive for anti-A₁. The strength of anti-A₁ reaction was 32 after incubation at 4°C. Strength of antibody demonstrates its significance in transfusion practices especially when the thermal amplitude of the reacting antibody is high. In this case, the reactivity was not enhanced at 37°C and showed weak reactivity at a titer of 8 while no reactivity at 16 and beyond. Cold

reactive anti-A₁ has been reported in the literature.¹⁶ The literature shows the prevalence of anti-A₁ among A₂ and A₂B to be 1–8% and 22–35% respectively.^{2–4} Chaudhari et al, (2008) has reported a case of IgG anti-A₁.¹⁷ Similarly, two other studies have shown hemolytic transfusion reaction due to anti-A₁.^{18,19} Development of anti-A₁ antibodies after allogeneic stem cell transplantation and organ transplantation has also been reported.^{7,20}

It has been reported that individuals with A₂B phenotype are more prone to develop anti-A₁ as compared to A₂. This could be explained on the basis of two observations. Firstly, individuals with A₂B have a smaller number of A antigens in comparison to A₂. Secondly, A₂B individuals possess *R101 allele more commonly than A₂ individuals (41% vs 1%) leading to the high frequency of A₂B phenotype.²¹

From a transfusion perspective, individuals with A₂ and A₂B should be transfused with identical blood types. However, due to its rarity especially A₂B, special attention shall be given if identical blood type is not available and the patient needs transfusion of packed red cells. These individuals can be transfused with O group packed red cells considering it the next compatible group.

A major limitation of this study may be the small sample size due to which no anti-A₁ antibody was demonstrated in A₂ individuals.

Conclusion

In conclusion the prevalence of anti-A₁ in A₂ and A₂B is rare. It is important to rule out the possibility of its wide range of thermal reactivity. Any discrepancy in these individuals should be resolved before blood/component transfusion.

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Disclosure

The authors report no conflicts of interest in this work.

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