European Journal of Clinical Pharmacy

Print ISSN: 2385-409X **Online ISSN**: Applied
Website: https://farmclin.com/

Original Research Article

Prevalence of A2 and A2B and Anti A1 Antibody in Blood Groups A and AB in Healthy Donors

Article History:

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Received: 03-11-2025
Revised: 17-12-2025
Accepted: 21-12-2025
Published: 30-12-2025

Abstract:

Background: The ABO blood group system is the most important blood group system in transfusion medicine. Within blood group A, subgroups A2 and A2B are clinically significant due to their potential association with anti-A1 antibody formation, which may lead to transfusion-related complications if not identified.

Objective: To determine the prevalence of A2 and A2B subgroups among healthy blood donors with blood groups A and AB and to evaluate the presence of anti-A1 antibodies in these subgroups.

Study Design: Cross-sectional study

Place and Duration of Study: Department of Pathology at Fatima Jinnah Medical University, in association with, Blood Bank, Sir Ganga Ram Hospital in Lahore from 30 May 2025 to 30 September 2025.

Methodology: Healthy blood donors (N = 174) were enrolled. ABO grouping and subtyping were carried out by standard tube agglutination techniques using anti-A, anti-B and anti-A1 lectin. Anti-A1 antibodies were screened by revers grouping with A1 reagent red cells. SPSS software was used for the data analyses, and Chi-square test was used for association with $p < 0.05$ was significant.

Results: The occurrence of A2 subgroup was 12.6% in blood group A donors and A2B subgroup was 16.1% in AB donors. The incidence of anti-A1 antibodies was 9.1% in A2 and 28.6% in A2B group, significantly higher in A2B subgroup ($p < 0.05$).

Conclusion: A2 and A2B subgroups are present in a considerable proportion of donors and are associated with anti-A1 antibody formation, particularly in A2B individuals. Routine ABO subtyping and antibody screening are recommended to improve transfusion safety.

Keywords: ABO blood group, A2 subgroup, A2B subgroup, anti-A1 antibody, blood transfusion, hemolytic reaction, blood bank screening.

INTRODUCTION

The ABO blood group system is the most significant blood group system in transfusion medicine and immunohematology, identified by Karl Landsteiner in the early 1900s (Ajmani, 2020; Kvržić, 2024; Schneider, 2024). It is characterised by the presence or absence of A and B carbohydrate antigens on the red blood cell surface and these antigens follow the Mendelian mechanism of inheritance (Varpit & Galama, 2022; Kim, 2024; Misevic, 2021). The antigens are involved in blood transfusion, organ transplant and mother-fetus immunological interactions (Gehrie et al., 2021). Abnormal blood grouping and incompatibilities between ABO blood types (A, B, AB and O) result in acute hemolytic transfusion reactions and potentially death making blood grouping and compatibility testing a critical part of transfusion praxis (Panch &

Montemayor, 2022; Gehrie et al., 2021).

While the ABO system is divided into four major blood groups (A, B, AB and O), blood group A is distinguished into several subgroups based on the amount and intensity of A antigen expression (Abegaz, 2021; Lv et al., 2023). The A1 and A2 subgroups are the most important. Red blood cells of A1 contain high amounts of A antigen, as a result of large conversion of H antigen on the cell surface into A antigen by A1 transferase enzyme (Jajosky et al., 2023). On the other hand, A2 displays lower A antigen expression and has a higher proportions of unmodified H antigen on its surface (Chopra et al., 2022). In group A individuals, it is estimated 80% are A1 and 20% are A2, but there is considerable variation between different ethnic and geographical groups (Mishra et al., 2020).

A1/A2 differentiation is important from a clinical point of view (Khanum et al., 2024). People with A2 or A2B subtypes can develop anti-A1 antibodies, which are typically naturally occurring IgM antibodies that react at temperatures less than 25°C (Chowdhury et al., 2022; Saboor et al., 2020). These antibodies are usually clinically insignificant but, rarely, they may react at 37°C and produce clinically significant hemolytic transfusion reactions (Panch & Montemayor, 2022). Anti-A1 antibodies can be naturally occurring or may be an acquired consequence of alloimmunization via transfusion or pregnancy with exposure of A1 and/or A1B red cells (Yazer et al., 2022). A2B people are more susceptible to anti-A1 antibodies than A2 people due to decreased A antigen and increased H antigen determinants (Mishra et al., 2020).

The frequencies of A2 and A2B subgroups vary between populations. In Saudi Arabia, the frequency of A2 (2.24%) and A2B (0.9%) groups have been reported to be low in blood donors (Abdelaziz et al., 2021). However, Indian studies have reported higher frequencies, with the A2 frequency from 3.3% to 14.8% across different populations (Khanum et al., 2024). In northern Pakistan, the prevalence of A2 subgroup has been reported to be as high as 13.08% among blood group A individuals (Jelani et al., 2020), while studies from central Punjab have reported A2 and A2B to be 2.19% and 3.2% respectively (Saad, 2023). These differences reflect the role of genetic and ethnic variations as well as population substructure in the distribution of ABO subgroups.

The significance of anti-A1 antibodies is the risk of transfusion reaction (Chowdhury et al., 2022). These are generally IgMs, reacting at room temperatures, but have been shown to cause hemolysis at 37°C, especially in sensitized individuals (Berentsen, 2020; Loriamini et al., 2024). In a study conducted in North Karnataka, the prevalence of anti-A1 antibodies reported in A2 and A2B individuals was 0.4% and 25% respectively, revealing a greater immune risk with A2B subgroup (Mishra et al., 2020). These reports highlight the importance of routine serological testing in blood banks, particularly prior to transfusions in patients with weak A subgroups.

Although essential for clinical practice, ABO subtyping and the presence of anti-A1 antibodies in blood donors are not usually tested in blood banks, especially in developing countries (Usmani et al., 2024; Afroz & Saleh, 2024). This could result in undiagnosed incompatibilities and a greater risk of transfusion reactions. Thus, population studies are required to inform aspects of transfusion practices and enhance blood safety.

Objective

To establish the A2 and A2B subgroups' frequencies among healthy blood donors with blood groups A and AB respectively and to assess the presence of anti-A1 antibodies in these subgroups. The results will help improve transfusion safety, raise awareness among healthcare professionals and blood banking professionals and establish guidelines for ABO subtyping in blood banking.

METHODOLOGY

This cross-sectional study will be carried out in the Department of Pathology, Fatima Jinnah Medical University, and Blood Bank, Sir Ganga Ram Hospital, in Lahore, between 30 May 2025 and 30 October 2025. A sample of 174 cases is required with 95% confidence and 5% margin of error (prevalence of subgroup A2 is 13.08%). Eligible participants will be healthy blood donors (male and female, 18-60 years old), with blood groups A and AB. Donors eligible for standard blood donation (body weight ≥45 kg, Hemoglobin ≥12.5 g/dL and negative screening for transfusion transmitted viruses) will be surveyed. Individuals with blood groups B and O, history of transfusion in the past 12 months, and positive screening for transfusion infectious diseases will be excluded. Following informed consent, 6 mL venous blood sample will be taken from each donor (3 mL in EDTA vial and 3 mL in plain serum vial). ABO-Rh blood grouping and ABO subtyping will be done by tube agglutination method using anti-A, anti-B and anti-A1 lectin antibodies. A2 and A2B participants will be tested for the presence of anti-A1 antibodies using reverse grouping with A1 reagent red cells by immediate spin method and the presence of agglutination will be noted. The results will be analyzed with SPSS. Categorical variables such as blood group, subgroups (A1, A2, A1B, A2B), sex and frequency of alloantibodies (anti-A1) will be presented as frequencies and percentages. Numerical variable, age, will be shown as mean ± standard deviation (SD). Variables will be adjusted and stratified by blood group (A/AB) and gender to account for possible effect modifiers. The Chi square test will be used to test for associations between categorical variables, once stratification is done. p-value will be taken as significant if $p<0.05$.

RESULTS

We studied 174 healthy blood donors. All samples were tested for ABO blood grouping and subtyping and anti-A1 antibodies. SPSS software was used for data

analysis and data presented in frequencies, percentage, means ± SD and the Chi-square for associations. p-value <0.05 was considered significant. The mean age of study participants was 32.8 ± 9.6 years (Table 1).

Table 1. Age Distribution of Study Participants (n = 174)

Variable	Mean ± SD
Age (years)	32.8 ± 9.6

Males were predominant among donors (64.4%) (Table 2).

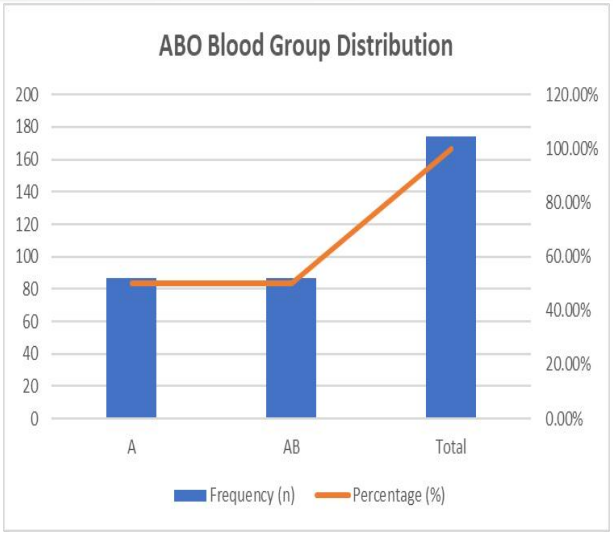
Table 2 Gender Distribution (n = 174)

GENDER	FREQUENCY (N)	PERCENTAGE (%)
MALE	112	64.4%
FEMALE	62	35.6%
TOTAL	174	100%

Equal distribution of blood groups A and AB was observed (Table 3).

Table 3. Blood Group Frequency (n) Percentage (%)

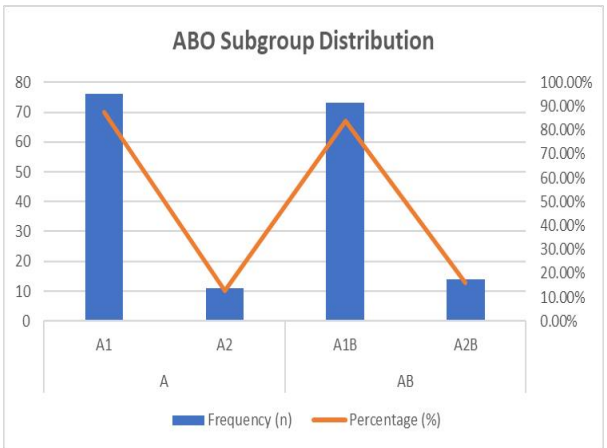
Blood Group	Frequency (n)	Percentage (%)
A	87	50.0%
AB	87	50.0%
Total	174	100%



Subgroup A2 was found in 12.6% of group A donors, while A2B was found in 16.1% of group AB donors. (Table 4).

Table 4 ABO Subgroup Distribution

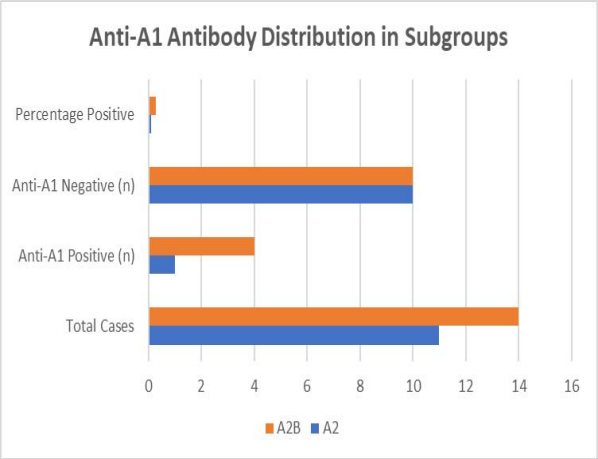
Blood Group	Subgroup	Frequency (n)	Percentage (%)
A	A1	76	87.4%
	A2	11	12.6%
AB	A1B	73	83.9%
	A2B	14	16.1%



Anti-A1 antibodies were significantly more frequent in A2B compared to A2 individuals.

Table 5: Anti-A1 Antibody Distribution in Subgroups

Subgroup	Total Cases	Anti-A1 Positive (N)	Anti-A1 Negative (N)	Percentage Positive
A2	11	1	10	9.1%
A2B	14	4	10	28.6%



No statistically significant association was observed between gender and ABO subgroups or anti-A1 antibody presence ($p > 0.05$).

Table 6: Gender-wise Association of Subgroups and Anti-A1 Antibody (Chi-square Test)

Variable	Category	Positive Cases	P-Value
A2/A2b Distribution	Male vs Female	Not significant	>0.05
Anti-A1 Antibody	Male vs Female	Not significant	>0.05

A statistically significant association was observed between A2B subgroup and higher frequency of anti-A1 antibody ($p < 0.05$).

Table 7: Association Between Blood Subgroups and Anti-A1 Antibody (Chi-square Test)

GROUP	ANTI-A1 POSITIVE (%)	P-VALUE
A2	9.1%	
A2B	28.6%	<0.05

DISCUSSION

This study aimed to determine the frequencies of A2 and A2B subgroups among healthy donors and of anti-A1 antibodies among these subgroups. The results illustrate the presence of weak subgroups among blood group A in a considerable number of donors with frequencies of 12.6% for A2 and 16.1% for A2B donors. This study underscores the significance of subtyping ABO blood groups in transfusion medicine, especially in areas lacking detailed serological screening (Usmani et al., 2024).

The frequency of the A2 subgroup seen in this study (12.6%) is similar to studies from northern Pakistan where frequencies as high as 13.08% have been reported and higher than studies from Saudi Arabia that report 2.24% prevalence. This difference in frequency is attributed to ethnic variations and genetic diversity. Likewise, variations in A2 prevalence (3.3-14.8%) in India are in line with our findings. These variations may guide transfusion safety policies in the respective regions (Khanum et al., 2024).

Here, the prevalence of A2B subgroup was found to be 16.1% which is comparatively higher than other reports, such as central Punjab (3.2%) and Saudi Arabia (0.9%). But such higher frequencies have been reported in some Indians and other South Asians, and suggest considerable variation in the expression of A2B among South Asians (Kumar et al., 2023). Increased prevalence of A2B in this study might have resulted from population-specific genetic variants and suggests the importance of close immunohematological testing of the AB donors (Mishra et al., 2020).

Another important observation in this study is the high frequency of anti-A1 antibodies in A2B individuals (28.6%) and a low frequency in A2 individuals (9.1%) (p-value < 0.05). This observation aligns with earlier reports that A2B individuals have a higher likelihood of forming anti-A1 antibodies because of lower amounts of A antigens expressed and exposure of H antigens - potential targets for the immune system (Mishra et al., 2020). Similarly, Mishra et al. (2020) also demonstrated an increased tendency to develop anti-A1 antibodies in A2B individuals, suggesting its immunological sensitivity.

Although anti-A1 antibodies are often naturally occurring IgM antibodies which are not clinically significant at room temperature, they can be thermostable and react at 37°C in vivo, highlighting their importance in transfusion medicine. Occasional cases of hemolytic transfusion reactions caused by anti-A1 antibodies have been reported, highlighting the need for the detection of these antibodies during pre-transfusion workup. Chowdhury et al. demonstrated hemolysis in a patient due to cold-reactive anti-A1 antibodies, indicating the possibility of severe clinical disease if and when they are present.

This study also found no association between gender and the distribution of ABO subgroups or anti-A1 antibody development ($p > 0.05$), suggesting gender variables do not play a significant role in ABO subgroup distribution and anti-A1 antibody development. This observation indicates that ABO subgroup expression and antibody formation are likely to be due to genetic and immunological factors, rather than sex or geographical factors, which is in line with

the literature reporting on ABO expression.

These results stress the value of ABO subtyping in blood group A and AB donors in blood transfusion laboratories. Routine subtyping is not performed in many developing countries, such as Pakistan, which can result in unanticipated incompatibilities and transfusion complications. Adopting anti-A1 lectin and antibody screening can play an important role in improving the safety of transfusions, particularly in tertiary care hospitals where complex transfusion requirements are frequent (Afroz & Saleh, 2024).

In conclusion, this study supports worldwide evidence that although A2 and A2B subgroups account for lesser frequencies than major ABO blood groups, they have disproportionate clinical significance due to their role in the development of anti-A1 antibodies and risk of hemolysis. Hence, it is highly advisable to include ABO subtyping in blood bank practices to ensure better transfusion safety and outcomes.

CONCLUSION

The current study has shown the importance of ABO subgroup variations in apparently healthy blood donors, especially A2 and A2B subgroups in blood groups A and AB respectively. This research shows A2 and A2B are not uncommon variants and are capable of inducing anti-A1 antibodies, particularly in A2B. The increased frequency of anti-A1 antibodies in A2B phenotype highlights its greater immunohematological importance and transfusion risk. While these antibodies are usually non-clinical, their occasional ability to react at body temperature may cause hemolytic transfusion reactions when inappropriate blood is transfused. So ABO subtyping and anti-A1 antibody screening should be part of routine blood banking procedures. This can boost transfusion safety, reduce complications and ensure optimal patient outcomes. The research also highlights the need for regional data for effective transfusion practice and standardizing laboratory screening protocols.

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