

Original Research Article

PREVALENCE AND CLINICAL IMPLICATIONS OF ANTI-A₁ ANTIBODIES IN A₂ AND A₂B BLOOD GROUP INDIVIDUALS: A FIVE-YEAR RETROSPECTIVE ANALYSIS

Agrawal Akanksha¹, Shigli Nahida A², K B Lakshmidevi³

¹Department of Pathology, SDM College of Medical Sciences and Hospital, Dharwad, India.

²Assistant Professor, Department of Pathology, SDM College of Medical Sciences and Hospital, Dharwad, India.

³Department of Pathology, SDM College of Medical Sciences and Hospital, Dharwad, India.

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Corresponding Author:

Dr. Shigli Nahida A,
Assistant Professor, Department of
Pathology, SDM College of Medical
Sciences and hospital, Dharwad,
Karnataka, India..
Email: nahidaafshan@gmail.com

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ABSTRACT

Background: The ABO blood group system includes subtypes of A, with A₁ and A₂ comprising the majority of A blood type. While both subtypes react with anti-A antibodies, only A₁ reacts with anti-A₁ antibodies. Some A₂ and A₂B individuals may develop anti-A₁ antibodies, which, if reactive at 37°C, can cause hemolysis and lead to hemolytic transfusion reactions. The aim was to determine the prevalence of A₂ and A₂B subtypes and the occurrence of anti-A₁ antibodies among A₂ and A₂B blood donors.

Materials and Methods: This retrospective study was conducted over 5 years at a tertiary care hospital in northern Karnataka. Blood grouping of 37,085 donors was performed using gel card technology. A and AB group samples were further tested with anti-A₁ lectin to identify A₁, A₂, A₁B, and A₂B subtypes. A₂ and A₂B samples were tested for Anti A₁ antibodies at room temperature and at 37°C.

Results: Of 13,147 A antigen donors, A₂ was found in 0.43% and A₂B in 2.45%. Anti-A₁ antibodies were detected in 14.03% of A₂ and 17.9% of A₂B individuals. Only two A₂B donors showed clinically significant Anti A₁ antibodies reactive at 37°C.

Conclusion: Although rare, clinically significant anti-A₁ antibodies can occur, especially in A₂B individuals. Routine screening in A₂ and A₂B donors is essential to ensure safe transfusion.

Keywords: ABO blood group system, A blood group, A₂ and A₂B subtypes, Anti A₁ antibodies, Cold agglutinin.

INTRODUCTION

A antigen is one of the major basis of ABO blood group system discovered by Landsteiner in 1901. Subtypes of A include A₁, A₂, A₃, A_x, A_{end}, A_m, A_y & A_{el}. Two major subtypes are A₁ and A₂ which differ from each other and account for almost 99% of all group A individuals.^[1] Both A₁ and A₂ subtypes react strongly with monoclonal anti-A antibodies however, A₁ subtypes react with anti-A₁ antibodies while A₂ subtypes do not. Anti- A₁ antibodies are atypical cold agglutinins found in the sera of some A₂ and A₂B subgroup individuals and they become clinically significant at 37°C leading to hemolysis of A₁ red cells. Therefore, testing and

knowing their prevalence is important in order to categorize the blood subtypes for compatible and safe transfusion practice which is done using lectin derived from seeds of *Dolichos biflorus*.^[2]

Few studies have been undertaken till now, which determine the prevalence of anti A₁ antibody in A₂ and A₂B subgroups. This study thus was conducted with an aim to determine the occurrence of A₂ subtypes in individuals having A antigen in their blood as well as to determine the prevalence of anti-A₁ antibody in A₂ and A₂B subgroups in donor population to decipher the clinical implications of this antibody.

MATERIALS AND METHODS

This retrospective study was conducted in the blood centre of a tertiary care hospital in Karnataka for a period of 5 years from January 2019 to December 2023. A total of 37085 blood donor samples were analyzed. Blood grouping was performed using Gel card technique for both forward and reverse grouping for all donors and verified. Samples of group A and AB were further tested with anti-A₁ lectin by tube method and then classified as A₁, A₂, A₁B and A₂B subgroups. A₂ or A₂B subgroup samples were tested with pooled A₁ cells to detect

the presence of anti-A₁ at room temperature (RT) 22°C and 37°C. Agglutination reactions are graded according to AABB standards.^[3] Data was tabulated and analysed using appropriate statistical methods.

RESULTS

A total of 37085 donors were analysed over a period of 5 years from January 2019 to December 2023. Of the total donors, 13147 (35.45%) donors had A antigen based blood groups (A₁, A₂, A₁B, A₂B) as given in the [Table 1].

Table 1: Distribution of blood donors having A antigen

Blood Group/year	2019	2020	2021	2022	2023	Total
A1	2377	1819	1874	1742	2110	9922
A2	14	7	8	15	13	57
A1B	684	482	489	558	632	2845
A2B	92	55	48	69	59	323
Other Groups	5736	4005	4452	4667	5078	23938

Of the total 13147 donors with A antigen, A₂ antigen was found in 380 donors (2.89%). Donors having A₂ blood group were 57 (0.43%) and A₂B were 323 (2.45%). Prevalence of A₂ antigen in AB blood group (10.20%) was more than that of A₂ in A blood group (0.57%).

Anti-A₁ antibodies were found in 8 donors with A₂ blood group of which none were clinically significant (reactive at 37 °C) and similarly, anti-A₁ antibodies were found in 58 donors of A₂B blood group of which only in 2 donors they were clinically significant (reactive at 37 °C) as depicted in [Figure 1 & 2].

The prevalence of Anti-A₁ antibody in A₂B was higher as compared to A₂.

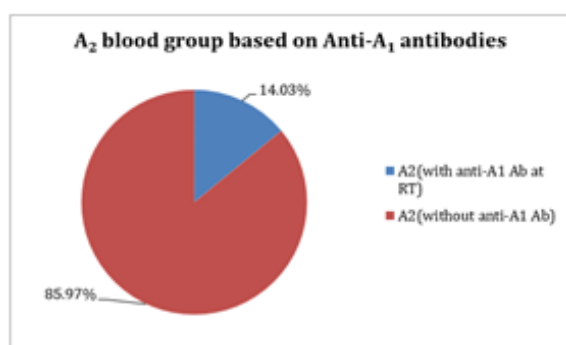


Figure 1: A₂ blood group based on Anti-A₁ antibodies

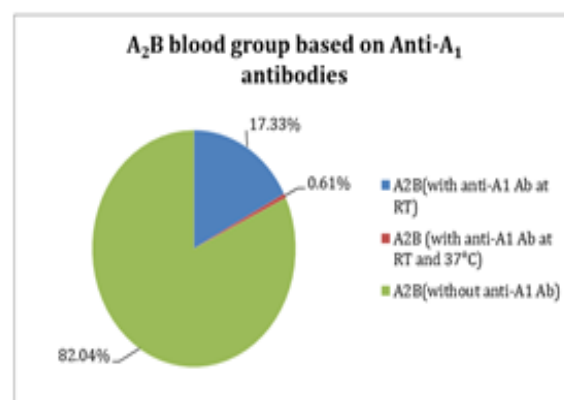


Figure 2: A₂B blood group based on Anti-A₁ antibodies

DISCUSSION

Major blood groups under the ABO system are A, B, AB and O. Prevalence of A blood group in Indian population is 22-30%. Blood group 'A' has numerous subtypes of which A₁ and A₂ form the bulk.^[2,4,5]

The prevalence of A₂ and A₂B amongst blood group A individuals in the present study was found to be 0.43% and 2.45% respectively. This was largely concordant with other studies as shown in [Table 2].

Table 2: Prevalence of A₂ and A₂B amongst all individuals with A antigen in blood across various studies.

Study	A ₂ (%)	A ₂ B (%)
Giriyani et al. ^[2]	0.8	2.5
Shastri et al. ^[4]	1.47	2.15
Mishra et al. ^[6]	1.28	1.18
Sharma et al. ^[7]	5.7	2.4
Bangera et al. ^[8]	1.12	1.88
Present study	0.43	2.45

In this study prevalence of A₂B in AB blood group was higher than the prevalence of A₂ in A blood

group which was concordant with the other studies listed in [Table 3].

Table 3: Prevalence of A2 antigen in A blood group and in AB blood group respectively in various studies

Study	A ₂ (%)	A ₂ B (%)
Giriyan et al. ^[2]	1.1	10.3
Shastri et al. ^[4]	1.85	10.5
Mishra et al. ^[6]	1.6	6.1
Sharma et al. ^[7]	8	8.6
Bangera et al. ^[8]	1.3	12.7
Present study	0.57	10.2

One question which always remains is what exactly do we mean by A₁ and A₂ and what they are. There are many explanations based on various theories and hypothesis provided in literature. However, A₁ blood group is formed due to A₁ gene which leads to conversion of most of the H precursor substance to A₁ antigen, whereas, A₂ gene produces lesser number of A₁ antigenic sites.^[10,11]

A₁ adults have approximately 0.8×10^6 antigen sites per red cell, whereas A₂ adults have 0.24×10^6 antigen sites per red cell. The lesser number of sites on A₂ adults is comparable to the number of antigen sites per red cell seen in A₁ neonates, i.e., $0.25-0.37 \times 10^6$. Hence, newborn red cells may not or weakly react with anti-A₁ reagents.^[4,12,13]

Qualitative difference between A₁ and A₂ blood groups also exists. Both branched and unbranched chains of H precursor are converted to A₁ antigen by A₁ gene. On the other hand, A₂ gene converts the unbranched chains and only a few branched chains of H precursor to A₁ antigen. In few cases of A₂ blood group, conversion of branched H precursor does not occur at all. It is in these individuals that Anti A₁ antibodies are hence, formed.^[10,11]

Anti A₁ antibodies are IgM cold-reacting antibodies, usually reacting at temperatures of <37°C.^[2,4,5,9]

A comparison of prevalence of Anti A₁ antibody in A₂ and A₂B blood groups with other studies is given in [Table 4].

Table 4: A comparison of prevalence of Anti A1 antibody in A2 and A2B blood groups with other studies

Study	Anti A ₁ in A ₂ (%)	Anti A ₁ in A ₂ B (%)
Shastri et al. ^[4]	1.8	3.75
Saboor et al. ^[5]	0	3.1
Mishra et al. ^[6]	0	23.07%
Das et al. ^[9]	1.9	19.4
Present study	14.03	17.9

Prevalence of Anti A₁ antibodies is more in A₂B as compared to A₂. B enzyme transferase has a strong ability to convert H structures to B antigen over co-existing A₂ enzyme. Based on the potency of A₂ gene, sometimes there will be no conversion of branched H precursor to A₁, thereby producing Anti A₁ antibodies more in A₂B than in A₂ blood group.^[11]

A₂ and A₂B units without Anti A₁ antibodies can be transfused to either A₁ or A₂ individuals. A₂ and A₂B units with Anti-A₁ antibodies which do not show agglutination at 37°C can also be considered for transfusion to either A₁ or A₂ subtype individuals. On the other hand, A₂ and A₂B units with anti-A₁ antibody reactive at 37°C should be transfused only to A₂ and A₂ B individuals.

CONCLUSION

This study highlights the prevalence of A₂ and A₂B subgroups among blood donors with A antigen. A higher occurrence of anti-A₁ antibodies is noted in A₂B individuals compared to A₂. While most of these antibodies are cold-reacting and clinically insignificant, very rarely a small number may demonstrate reactivity at 37°C, which is of clinical relevance. Identifying A subgroups and testing for anti-A₁ antibodies is essential to ensure compatibility and safe transfusion. Routine screening, especially in A₂ and A₂B individuals, for

anti-A₁ antibodies is crucial not only for general transfusion safety but also in high-risk settings. In neonates, where red cell antigen expression is less and immune system is still developing, the blood group can be misinterpreted as A₂. This hence, should be confirmed after 6 months of age. In cardiac surgeries and other critical care scenarios, where patients are hemodynamically unstable and may require urgent blood, transfusions are carried out at cold temperatures and since these Anti A₁ antibodies are cold reacting, they may pose a major threat. This testing hence explains transfusion protocol and also ensures safe blood transfusion.

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