Section: Pathology

Original Research Article

PREVALENCE AND THERMAL REACTIVITY OF ANTI-A1 ANTIBODIES IN A2 AND A2B INDIVIDUALS AT A TERTIARY BLOOD CENTRE

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ABSTRACT

Background: In the ABO blood group system, A1 and A2 are subgroups of A, while A1B and A2B are subgroups of AB. Some individuals with A2 and A2B phenotypes may develop anti-A1 antibodies, which may have clinical relevance in transfusion settings. **Aim:** To determine the frequency of A and AB subgroups and the prevalence of anti-A1 antibodies in A2 and A2B individuals among blood donors and recipients. **Materials and Method:** Blood samples from donors and recipients were typed for ABO (cell and serum grouping) and Rh D over one year (May 2024–April 2025). Individuals with A and AB groups were further subtyped using anti-A1 lectin into A1, A2, A1B, and A2B. A2 and A2B subgroups were screened for anti-A1 antibodies in serum. Thermal reactivity was assessed using A1 red cells at 4°C, 22°C, and 37°C. **Results:** Out of 25,512 samples, 5,310 (20.81%) were group A and 1,915 (7.51%) were group AB. In group A, 4,268 (80.37%) were A1 and 1,042 (19.63%) were A2. In group AB, 1,538 (80.31%) were A1B and 377 (19.69%) were A2B. Among A2 individuals, 43 (4.12%) were anti-A1 positive, while in A2B, 21 (5.57%) were anti-A1 positive. Most reactions occurred at 4°C and 22°C; however, 4 (9.3%) A2 and 2 (9.52%) A2B samples showed reactivity at 37°C, suggesting potential clinical significance. **Conclusion:** Anti-A1 antibodies are present in a small proportion of A2 and A2B individuals and may lead to discrepancies in reverse grouping. Although antibodies reactive at 37°C were rare, their presence suggests potential clinical significance. Routine screening may not be necessary in all cases; however, subgrouping and anti-A1 testing should be considered in cases of ABO discrepancy or suspected transfusion incompatibility to ensure safe transfusion practices.

INTRODUCTION

The International Society for Blood Transfusion (ISBT) recognises a total of 346 blood group antigens, categorised into 36 blood group systems.48 of these antigens have yet to be assigned to any blood group system.[1] ABO blood group antigens are present not only on red blood cells but also on platelets, lymphocytes, and cells in various tissues like the vascular endothelium, intestine, cervix, urethra, and mammary glands.[2]

The ABO blood group system, one of the most significant and well-known human blood group systems, is crucial in transfusion medicine, organ transplantation, and immunology. It is based on the presence or absence of two main antigens, A and B, found on the surface of red blood cells. These antigens determine an individual's blood group, which can be classified into four main types: A, B, AB, and O.[1,2] Within the ABO system, A2 and A2B are less common subtypes of A and AB blood groups, respectively. These subtypes have specific genetic characteristics that distinguish them from the more

common A1 phenotype. The differences between the A1 and A2 phenotypes are quantitative (in terms of how much of the antigen is expressed) and qualitative (in terms of the chemical structure of the A antigen).[2-4]

Specifically, A1 individuals have more A antigen on their red blood cells, including specific antigenic determinants (Ac and Ad). A2 individuals lack Ac and Ad determinants and express only Aa and Ab. Because of this difference, A2 and A2B individuals may form anti-A1 antibodies that react against A1 cells. This immune response may have clinical significance, particularly in blood transfusion settings, as anti-A1 antibodies can cause complications when A2 or A2B individuals are exposed to A1 red blood cells. Anti-A1 antibodies are typically cold-reacting agglutinins, reacting at 4°C and 22°C but usually not at 37°C, meaning they typically react at room and cold temperature (4 and 22°C), but not at the body temperature of 37°C.[5,6]

The reported prevalence of anti-A1 antibodies varies widely across studies, with a lower frequency in A2 individuals and a comparatively higher frequency in A2B individuals.7 The presence of these antibodies is more common in A2B individuals (about 25%) compared to A2 individuals (about 0.4%). These antibodies can bind to A1 red blood cells, causing hemolysis. This can lead to hemolytic reactions during blood transfusions, especially if an A2 or A2B individual receives A1 red blood cells.[8] However, data on the prevalence and clinical significance of anti-A1 antibodies in A2 and A2B individuals in the Indian population remain limited. Therefore, the present study was undertaken to determine the frequency of A and AB subgroups and to assess the prevalence and clinical significance of anti-A1 antibodies in A2 and A2B individuals in a tertiary care blood centre.

MATERIALS AND METHODS

This study was conducted in the department of pathology from May 2024 to May 2025 in a tertiary blood centre. Ethical permission was obtained from the institute's ethical committee before the initiation of the study. Informed consent was obtained from all the donors and patients.

Inclusion and Exclusion Criteria

All donors and patients undergoing blood grouping at the blood centre during the study period were included. Donors deferred from blood donation, individuals with a history of previous transfusion reactions were excluded from the study.

Methods

A total of 25512 blood samples of both donors and patients were included in our study. ABO blood grouping was done using the test tube technique. Forward grouping was done using monoclonal antisera anti A, anti B, anti D and reverse grouping was done using in house prepared pooled RBC A cells, B cells, and O cells. Blood samples of groups A and AB were further tested with anti A1 lectin to classify them into A1, A2 and A1B, A2B subgroups, Further, the plasma of A2 and A2 B subgroups was tested with A1 red cells to confirm the presence of anti A1 antibodies. The thermal amplitude of anti A1 antibodies was determined by keeping the test tubes at 4°C, 22°C, and 37°C.9 The methodology of sample testing is shown in Figure 1.

Statistical Analysis: Categorical variables such as blood group distribution, subgroup frequency, and prevalence of anti-A1 antibodies were expressed as frequencies and percentages. The prevalence of anti-A1 antibodies in A2 and A2B subgroups was calculated with respect to their total subgroup population. Thermal reactivity patterns were also expressed as proportions. No inferential statistical tests were applied.

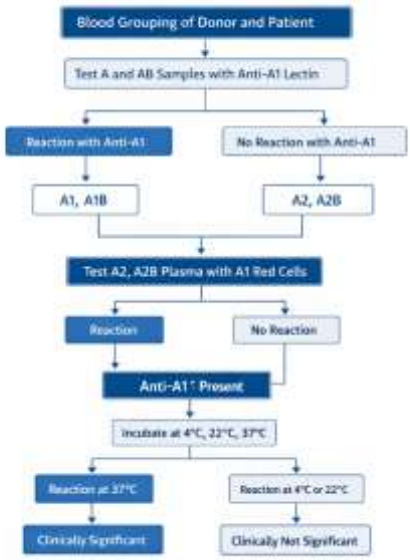

Figure 1: Determination of Anti-A1 Antibodies in A2 and A2B Subgroups

RESULTS

A total of 25512 blood samples of both patients and donors were analysed to know the frequency of different types of blood groups as mentioned in Table 1. Out of those, the blood group A were 5310 (20.81%), and AB were 1915 (7.51%). Among blood group A, 4268 (80.37%) were A1 and 1042 (19.63%) were A2. Among blood group AB, 1538 (80.31%) were A1B and 377 (19.69%) were A2B in Table 2, Figure 2, 3. Among A2 blood group subtypes, 43 (4.12%) were Anti A1 positive, 999 (95.87%) were Anti A1 negative and among A2B blood group, 21(5.57%) were Anti A1 positive and 356 (94.43%) were Anti A1 negative as mentioned in Table 3, Figure 4, 5. Among anti-A1 positive individuals, 9.3% (4/43) of A2 and 9.52% (2/21) of A2B samples showed reactivity at 37°C, suggesting potential clinical significance. When calculated over the total

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subgroup population, this corresponds to 0.38% (4/1042) and 0.53% (2/377), respectively, which is illustrated in Table 4, Figure 6, 7.

Table 1: Frequency of different types of blood group individuals

Blood group	Rh positive	Rh negative	Total	Percentage (%)
A	5213	97	5310	20.81
B	7449	124	7573	29.68
AB	1872	43	1915	7.51
O	10539	175	10714	42.00
Total	25073	439	25512	100

Table 2: Frequency of A subgroup types

Blood Group	Subgroup	Total (N)	Frequency (%)
A	A1	4268	80.37
	A2	1042	19.63
AB	A1B	1538	80.31
	A2B	377	19.69

Figure 2: ABO and Rh group of A2 (Shown in left) and A2B (Shown in right) blood group by Tube method, forward and reverse grouping

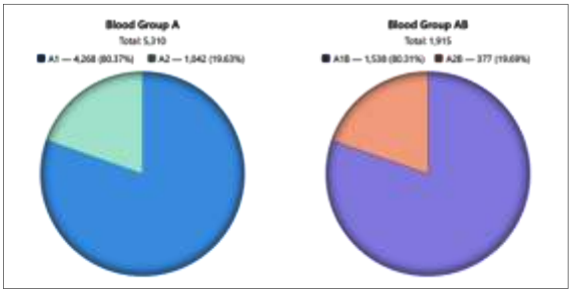

Figure 3: Pie chart shows the frequency of A and AB subgroups (A1, A2 as shown in left and A1B, A2B as shown in right)

Table 3: Prevalence of anti-A1 antibodies in A2, A2B blood group

Blood group (N)	ANTI A1 Ab positive (N)	Frequency (%)
A2 (1042)	43	4.12%
A2B (377)	21	5.57%

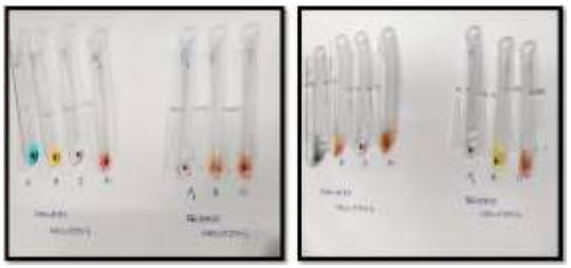

Figure 4: Results showing A2 positive (Shown in left) and A2B positive (Shown in right) with anti-A1 antibodies

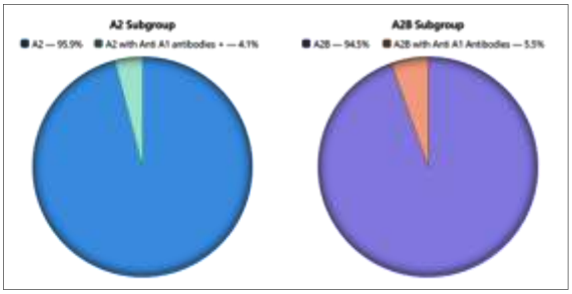

Figure 5: Pie chart shows the frequency of Anti A1 antibodies in A2 and A2B blood group

Table 4: Thermal reactivity pattern of anti-A1 antibodies among antibody-positive A2 and A2B individuals

Blood group (N)	Anti A1 antibodies which react at 4°C, 22°C only	Prevalence	Reaction at 37°C as well	Prevalence
A2 (1042)	39	90.7%	4	9.3%
A2B (377)	19	90.48%	2	9.52 %

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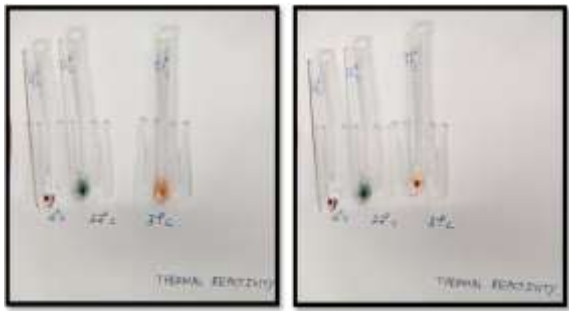

Figure 6: Thermal reactivity of A2B blood group with Anti A1 antibodies; Left side (Reaction at only 4°C, 22°C), Right side (Reaction at 4°C, 22°C and 37°C)

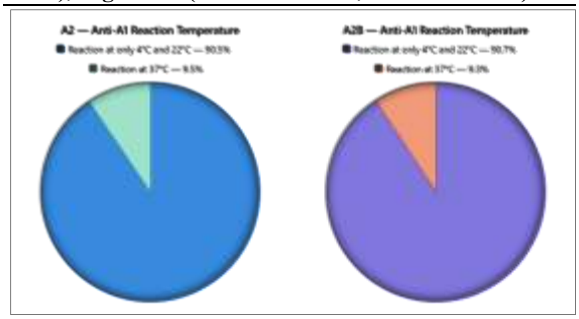

Figure 7: Pie chart shows the Prevalence of Thermal reactivity of A2 (Left side) and A2B (Right side) blood group with anti-A1 antibodies at 4°C, 22oC and 37 °C

DISCUSSION

The A and AB blood groups are divided into subgroups such as A1 and A2. These subgroups show differential reactivity with monoclonal anti-A reagents. To differentiate between these subgroups, an anti-A1 lectin reagent obtained from Dolichos biflorus seeds is used. The proportion of A2 among A group individuals in this study was 19.63%. This variation may be due to differences in the study population and inclusion criteria.[9]

In the present study, A2 constituted 19.63% of group A individuals, and A2B constituted 19.69% of group AB individuals. Direct comparison with other studies is limited, as reported prevalence varies depending on whether calculations are based on the total population or specific blood group subsets. The prevalence of anti-A1 antibodies observed in this study (4.12% in A2 and 5.57% in A2B) differs from classical literature reports, which describe a lower frequency in A2 and a higher frequency in A2B individuals.[10,11] This variation may be attributed to differences in population characteristics, sample size, inclusion of both donors and patients, and methodological differences in antibody detection. In this study, 4.12% of A2 and 5.57% of A2B individuals showed the presence of anti-A1 antibodies, respectively, which react at 4°C, 22°C and of these 4 (9.3%) and 2 (9.52%) samples show reactivity at 37 °C as well, respectively, which is clinically significant. However, clinically significant antibodies were rare when considered against the total population, indicating limited overall clinical impact. This study results slightly higher than the study by Shastry and Bhat et al., found anti A1 in 1.8% of A2 and 3.75% of A2 B individuals, but none of them was clinically significant.[12] Variations across studies may be due to differences in sample size, population demographics, methodology, and geographic diversity.

The relatively higher prevalence of anti-A1 antibodies in A2B individuals has been attributed in previous studies to genetic factors such as the R101 allele; however, no molecular analysis was performed in the present study. This allele is found in 41% of A2B individuals, compared to just 1% in A2 individuals.[13,14] A2 and A2B individuals sometimes show discrepancies in blood typing due to the presence of anti-A1 antibodies. These antibodies can cause mismatches or unusual reactions when transfused blood is tested.[15] Such discrepancies were observed in both A2 and A2B individuals in this study. The discrepancies caused by the presence of anti-A1 antibodies are usually seen during the reverse typing (which is the test where an individual's serum is mixed with known red blood cells). To differentiate from auto antibodies, check by using A2 pooled cells instead of A1 cells, as the anti-A1 antibodies specifically target A1 cells. Individuals with Anti-A1 antibodies are typically cold agglutinins, meaning they react at lower temperatures. These antibodies cause clumping (agglutination) when mixed with A1 red blood cells. Anti-A1 antibodies are generally not clinically significant when they react at 4°C, 22°C and are considered clinically significant when it reacts at 37°C.[16,17] The patients with clinically significant anti A1 antibodies should receive either A2 or O red cell units in case of A2 recipients, and A2 B recipients should receive AHG crossmatch compatible saline washed red cells of group A2 B or O.[15,18,19] This ensures that A2 blood components are safely transfused, providing good recovery and proper count increment in the recipient.[20]

CONCLUSION

Data on A2 and A2B subgroups and anti-A1 antibodies in the Indian population are limited. This study shows that A2 and A2B constitute a notable proportion of A and AB blood groups, with anti-A1 antibodies present in a small percentage of individuals. Although antibodies reactive at 37°C, indicating clinical significance, were rare, their presence cannot be overlooked. Routine screening may not be necessary in all cases; subgrouping of A and AB blood groups and testing for anti-A1 antibodies should be considered in cases of ABO discrepancy, incompatible crossmatch, or suspected transfusion reactions to ensure safe transfusion practices.

REFERENCES

1. Storry JR, Castilho L, Chen Q, Daniels G, Denomme G, Flegel WA, et al. International society of blood transfusion working party on red cell immunogenetics and terminology: report of

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the Seoul and London meetings. ISBT Sci Ser 2016;11:118–22. https://doi.org/10.1111/voxs.12280.
2. Hod EA, Spitalnik PF, Spitalnik SL. Carbohydrate Blood Groups. Rossi's Principles of Transfusion Medicine 2009:89–108. https://doi.org/10.1002/9781444303513.ch6.
3. Harmening DM, Manning BL, editor. Modern Blood Banking & Transfusion Practices. 7th ed. Philadelphia: F.A. Davis; 2019:119–148. Available from: https://www.scribd.com/document/444431449/Modern-Blood-Banking-Transfusion-Practices-7th-Ed-pdf (accessed April 1, 2026).
4. Cooling L. Chapter 12: ABO, H, and Lewis blood groups and structurally related antigens. In: Fung M, Grossman B, Hillyer CWC, editors. Technical Manual. 18th ed. Bethesda: AABB; 2014:290–315. Available from: https://bancodesangreucr.wordpress.com/wp-content/uploads/2016/03/chap12.pdf (accessed April 1, 2026).
5. Domen RE, Calero A, Keehn WH. Acute hemolytic transfusion reaction due to anti-A1. Lab Med 1988;19:739–40. https://doi.org/10.1093/labmed/19.11.739.
6. Northoff H, Wölpl A, Sugg U, Junginger W, Meinke J, Welter J, et al. An unusual sample of irregular anti-A1, probably causing an early delayed transfusion reaction. Blut 1986;52:317–21. https://doi.org/10.1007/bf00320795.
7. Bracey AW, Van Buren C. Immune anti-A1 in A2 recipients of kidneys from group O donors. Transfusion 1986;26:282–4. https://doi.org/10.1046/j.1537-2995.1986.26386209389.x.
8. Akkök ÇA, Haugaa H, Galgerud A, Brinch L. Severe hemolytic transfusion reaction due to anti-A1 following allogeneic stem cell transplantation with minor ABO incompatibility. Transfus Apher Sci. 2013;48(1):63–66. https://doi.org/10.1016/j.transci.2012.07.006
9. Mishra D, Prakash S, Sahoo D, Ray GK, Routray SS, Das PK, et al. Prevalence of A2and A2B subgroups along with anti-A1 antibody in patients and donor population and its clinical significance. J Appl Hematol 2020;11:112-5. https://doi.org/10.4103/joah.joah_15_20
10. Mahapatra S, Mishra D, Sahoo D, Bhusan B, Resident SJ. Medical Science. Worldwidejournals.com n.d. https://www.worldwidejournals.com/international-journal-of-scientific-research-(IJSR)/recent_issues_pdf/2016/March/March_2016_1492751044__59.pdf (accessed April 1, 2026).
11. Chaitanya Kumar IS, Yashovardhan A, Suresh B bua B, Verma A, Sreedhar Babu KV, Jothi Bai DS. The prevalence of A2 and A2B subgroups in blood donors at a tertiary care teaching hospital blood bank of Rayalaseema region: A pilot study. J Clin Sci Res 2012;1:50. https://doi.org/10.15380/2277-5706.jcsr.12.012.
12. Shastry S, Bhat S. Imbalance in A_2 and A_2B phenotype frequency of ABO group in South India. Blood Transfus 2010;8:267–70. https://doi.org/10.2450/2010.0147-09.
13. Voak D, Lodge TW. A possible explanation for the excess of A2B phenotypes observed in some populations. Vox Sang 1970;18:471–4. https://doi.org/10.1111/j.1423-0410.1970.tb01486.x.
14. Ogasawara K, Yabe R, Uchikawa M, Bannai M, Nakata K, Takenaka M, et al. Different alleles cause an imbalance in A2 and A2B phenotypes of the ABO blood group. Vox Sang 1998;74:242–7. https://doi.org/10.1046/j.1423-0410.1998.7440242.x.
15. Cooling L. ABO, H and Lewis structurally related antigens. In: Fung M, Grossman B, Hillyer C, Westhoff C, editors. Technical Manual. 18th ed. Bethesda: AABB; 2018. p. 295 7. Available from: https://bancodesangreucr.wordpress.com/wp-content/uploads/2016/03/chap12.pdf (accessed April 1, 2026)
16. Helmich F, Baas I, Ligthart P, Bosch M, Jonkers F, de Haas M, et al. Acute hemolytic transfusion reaction due to a warm reactive anti-A1: Strong Anti-A1 Agglutinins. Transfusion 2018;58:1163–70. https://doi.org/10.1111/trf.14537.
17. Preece J, Magrin G, Webb A, Akers C, Davis A. Transfusion medicine illustrated. A bloody mistake: unrecognized warm reactive anti-A1 resulting in acute hemolytic transfusion reaction. Transfusion 2011;51:914–5. https://doi.org/10.1111/j.1537-2995.2011.03064.x.
18. Fifth Edition. Requirements for transfusion. Gov.au n.d. https://www.safetyandquality.gov.au/sites/default/files/2023-02/requirements_for_transfusion_laboratory_practice_fifth_edition_2023.pdf (accessed April 1, 2026).
19. Hosseini-Maaf B, Hellberg A, Rodrigues MJ, Chester MA, Olsson ML. ABO exon and intron analysis in individuals with the AweakB phenotype reveals a novel O1v-A2 hybrid allele that causes four missense mutations in the A transferase. BMC Genet 2003;4:17. https://doi.org/10.1186/1471-2156-4-17.
20. Redfield RR, Parsons RF, Rodriguez E, Mustafa M, Cassuto J, Vivek K, et al. Underutilization of A2 ABO incompatible kidney transplantation: A2 incompatible kidney transplantation. Clin Transplant 2012;26:489–94. https://doi.org/10.1111/j.1399-0012.2011.01543.x.

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