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RESEARCH ARTICLE

Estimation of A2 subgroup in A and AB blood groups of human blood and its clinical significance

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Abstract: ***BACKGROUND:*** The clinical implications of anti A1 antibodies in individuals with A_2 and A_2B blood types remain a subject of debate. Although rare, a few case reports have documented clinically significant anti A1 antibodies that react at 37 °C and lead to haemolysis of donor red blood cells. This study therefore seeks to determine both the prevalence of the A_2 and A_2B subgroups and the clinical relevance of anti A1 antibodies within these populations. ***MATERIALS AND MATHODS:*** This cross-sectional observational study that was conducted at the RL Jalappa Blood Bank Centre. Blood group determination and differentiation between A1 and A2 subgroups. Blood group determination and differentiation between A1 and A2 subgroups was performed using the gold-standard test Column technology, based on the agglutination reaction with anti-A1 lectin. Serum samples from individuals with A2 and A2B subgroups was tested using A1 red cells to confirm the presence of anti-A1 antibodies. Further analysis was conducted to identify the type of anti-A1 antibody—whether IgM or IgG—and assess its clinical significance. ***RESULTS:*** A total of 4,000 blood samples from individuals with A and AB blood groups—comprising both patients and donors—were analysed. Among these, 3,150 were blood group A and 850 were group AB. Within the A group, 3,070 were subtype A_1 and 80 were A_2, while AB participants included 820 A_1B and 30 A_2B individuals Out of 3,000 donors, the prevalence of subgroups was 98.7% A_1, 1.3% A_2, 94.1% A_1B, and 2.9% A_2B. In the cohort of 1,000 patients, A_1 and A_2 accounted for 93.8% and 6.2%, respectively, while A_1B and A_2B represented 92.1% and 7.9%, respectively. No anti A_1 antibodies were detected in the serum of A_2 individuals, either donors or patients; similarly, none of the A_2B donors or patients exhibited anti A_1 antibodies. These antibodies showed no clinical significance, as they did not cause agglutination at 37 °C. ***CONCLUSIONS:*** To date, no investigations from Southern Indian state of Karnataka have explored the prevalence of A_2 and A_2B subgroups and the presence of anti A_1 antibodies in both donor and patient populations. Our study indicates that routine screening for anti A_1 Antibodies in individuals with A_2 or A_2B subgroups is a good practice. However, in transplantation settings, it remains mandatory to subtype A and AB blood groups and specifically check for anti A_1 antibodies.

Keywords: ABH blood group, Anti-A1 lectin, blood Group A, AB

INTRODUCTION

More than a century after the discovery of the ABO blood group system, immunohematology still encounters challenges when identifying ABO subtypes and weaker antigen variants. The antigenic properties of the ABO blood groups stem from specific carbohydrate structures displayed on the surface of red blood cells—structures that also occur in epithelial cells, vascular endothelial cells, and platelets. [1] Due to polymorphisms in the ABO genes, reduced expression of A or B antigens occurs on red blood cells, giving rise to A and AB subgroups. The two most common subgroups, A_1 and A_2, are distinguished both quantitatively—A_1 cells carry significantly more antigen sites than A_2—and qualitatively, with distinct antigen structures [2] Both A_1 and A_2 red cells exhibit strong agglutination with monoclonal anti-A reagents. However, they can be differentiated serologically using lectin derived from *Dolichos biflorus* (anti-A_1 lectin), which agglutinates only A_1 cells—thus distinguishing the A_1 and A_2 subgroups.

The *Dolichos biflorus* lectin agglutinates only A_1 red cells and does not affect A_2 cells. Studies in India reveal that within blood group A, A_1 and A_2 subgroups occur at frequencies of approximately 98.14% and 1.07%, respectively; among AB individuals, A_1B and A_2B subgroups are found in about 89.28% and 8.99%, respectively.[3]Anti-A_1 antibodies frequently manifest as atypical cold agglutinins—reacting at room temperature—and are sometimes detected in the serum of A_2 individuals. These IgM-type antibodies bind red cells under cooler conditions, though they typically have diminished or no activity at 37 °C or Anti-A_1 antibodies often present as atypical cold agglutinins, reacting at room temperature, and are sometimes detected in the serum of A_2 or A_2B individuals who do not express the A_1 antigen [4].

Only around **0.4 %** of individuals with an A_2 blood group—and **25 %** of those with A_2B—harbour anti-A_1 antibodies in their serum, which may become clinically significant if they react at 37 °C, potentially causing haemolysis of A_1 red cells [4-6]

Adult A_1 red cells carry approximately $\mathbf{0.8 \times 10^6}$ antigen sites per cell, whereas adult A_2 cells have about $\mathbf{0.24 \times 10^6}$, a level similar to that found in A_1 neonatal cells, which range between $\mathbf{0.25–0.37 \times 10^6}$ sites per cell, New born red cells may show little to no reaction—or only weak agglutination—with anti-A_1

reagents, due to their underdeveloped antigen expression at birth.[7] In southern part of India, only a handful of studies have examined the prevalence of A_1 and A_2 subgroups among blood donors, with few involving patients. To address this gap, our study aimed to determine the prevalence of A_1 and A_2 subgroups—as well as anti-A_1 antibodies within both donor and patient populations for A and AB blood groups, and to evaluate whether any of these antibodies exhibit clinical significance.

Objectives:
The present study was taken up with the following aims and objectives

- **To assess the distribution of blood subgroups A_1, A_2, A_1B, and A_2B among the population in Kolar District, Karnataka, India.**
- **To investigate the clinical significance of these blood subgroups in medical practice.**

Materials and Methods:
This cross-sectional observational study was conducted at the RL Jalappa Blood Bank Centre**.** Blood group determination and differentiation between A_1 and A_2 subgroups was performed using the gold-standard test Column technology, based on the agglutination reaction with anti-A_1 lectin. Serum samples from individuals with A_2 and A_2B subgroups was tested using A_1 red cells to confirm the presence of anti-A1 antibodies. Further analysis was conducted to identify the type of anti-A1 antibody—whether IgM or IgG—and assess its clinical significance.

Sample size calculation will be based on the following parameters:
Margin of error (MOE): 9.6%

- **Expected distribution proportion (p):** 30% of the blood donor population
- **Confidence interval:** 95%
- **Software used:** OpenEpi Info

The sample size (n) will be calculated using the formula:
$n=N\times XX+N-1n = \frac{N \times X}{X + N - 1}$
Where:
$X=Z\alpha/22\times p\times(1-p)MOE2X = \frac{Z_{\alpha/2}^2 \times p \times (1 - p)}{MOE^2}$
Here, $Z\alpha/2=1.96Z_{\alpha/2} = 1.96$, corresponding to a 95% confidence level. pp is the estimated sample proportion, and NN is the total population size.
A total of **4000** samples comprising 3000 eligible donors and 1000 patients was included in the study.

Inclusion Criteria

- Donors who meet the eligibility requirements as per the Drugs and Cosmetics Act.
- Individuals who have provided written informed consent.
- Patients with blood groups A and AB.

Exclusion Criteria

- Donors with irregular red cell antibodies.
- Sero positive donors.
- Donors and patients who have not provided consent.
- Patients of other blood groups.
- Neonates.

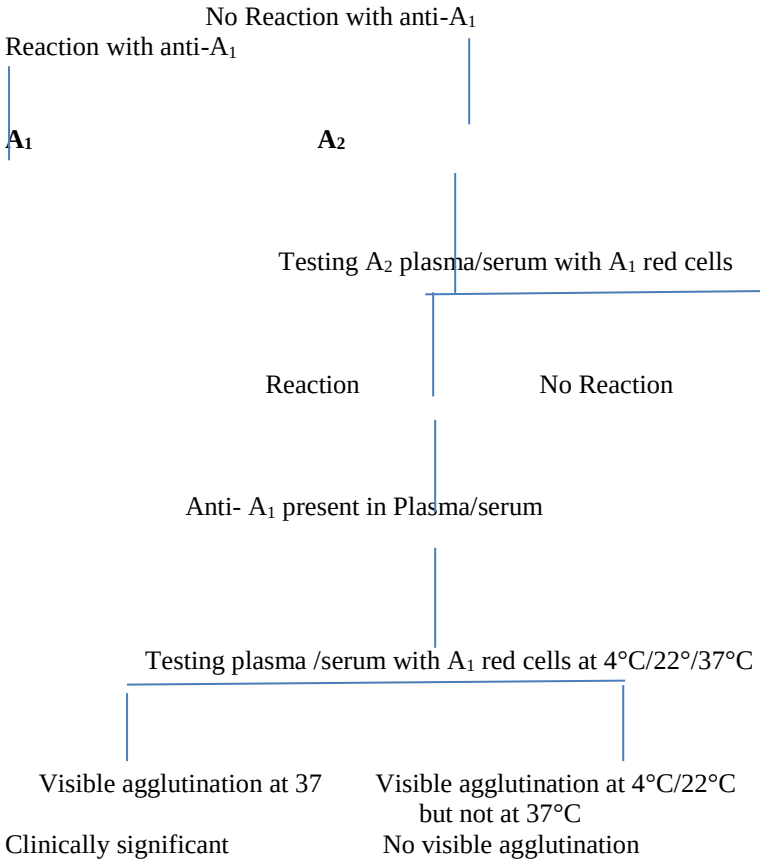
Page 5

Study Procedure
This is Cross sectional observational study was conducted at RL Jalappa blood bank centre.
From March 2023 to March 2025 which is a tertiary care centre of South Eastern state of Karnataka, India. A total of 4000 blood samples of both donors and patients of A and AB blood groups were included in our study. Thirty-two blood samples of the neonate with four A_2 and threeA_2B were excluded from the study. This is because ABO antigens are not fully developed at birth, and red cells of neonates of the A1 blood group may not react or react weakly with anti-A_1.

RESULT:

Study Protocol
Performance of blood grouping among the donors and the patients

Estimation of A and AB blood samples of the donors and patients
With anti- A_1lectin



Flowchart 1: Determination of A_1 and A_2 and significance of anti-A_1 antibody Page 6

A total of 4000 blood samples from individuals with A and AB blood groups—comprising both patients and donors—were analysed. Among these, 3,150 were blood group A and 850 were group AB. Within the A group, 3,070 were subtype A_1 and 80 were A_2, while AB participants included 820 A_1B and 30 A_2B individuals (see Table 1).

Out of 3,000 donors, the prevalence of subgroups was 98.7% A_1, 1.3% A_2, 94.1% A_1B, and 2.9% A_2B. In the cohort of 1,000 patients, A_1 and A_2 accounted for 93.8% and 6.2%, respectively, while A_1B and A_2B represented 92.1% and 7.9%, respectively.

No anti-A_1 antibodies were detected in the serum of A_2 individuals, either donors or patients; similarly, none of the A_2B donors or patients exhibited anti-A_1 antibodies. These antibodies showed no clinical significance, as they did not cause agglutination at 37 °C (see Table 2).

DISCUSSIONS

The A and AB blood groups can be further classified into major subgroups, such as A_1 and A_2. These subgroups react similarly with monoclonal anti-A reagents; however, distinguishing between them requires the use of anti-A_1 lectin, derived from *Dolichos biflorus* seeds. In this study, the prevalence of the A_2 subgroup was found to be (2.6%) and (3.6%) among individuals with blood group A and among those with blood group AB. These findings are consistent with a previous study conducted in South India by Shamee S et al.[4] Other research has reported the prevalence of A_2 and A_2B among blood donors to range between 4.1%–5.8% and 19.2%–31.5%, respectively.[8,9] In the current study, the distribution of A_1 and A_2 within the A blood group closely aligned with results from Giriyan et al., whereas the prevalence of A_1B and A_2B within the AB group was slightly higher[10] This increased prevalence of the A_2B subgroup compared to A_2 may be attributed to the suppressive effect of a strong B gene on A_1 antigen expression.[11]

In our study population, the prevalence of A_1 and A_2 subgroups was nearly identical among both donors and patients. Individuals with the A_2B phenotype are more likely to produce anti-A_1 antibodies than those with the A_2 phenotype, due to the relatively lower expression of A antigens on A_2B red blood cells.[12] In this study, there is no anti-A_1 antibodies were detected in A_2 blood group donor or patients similarly with A_2b blood group donor and patients[4] The higher incidence of anti-A_1 in A_2B individuals has a genetic basis—specifically, the presence of the *R101 allele, found in 41% of A_2B individuals compared to only 1% of those with A_2.

[1]This allele appears in A_1 (*R101/*O) and A_2 (*R101/*B) individuals as a heterozygous combination with either the O or B allele. The presence of anti-A_1 antibodies in A_2 and A_2B individuals can sometimes lead to blood group discrepancies, primarily during reverse typing. [1] In our study, such discrepancies were observed in three A_2B patients, while none of the A_2 individuals exhibited anti-A_1 antibodies. [14] These discrepancies are typically resolved using pooled A_2 cells instead of A_1 cells during reverse typing.

Anti-A1 antibodies are usually cold-reacting and agglutinate at room temperature; however, in rare cases, they may react at 37°C and cause significant haemolysis of A_1 cells.[15,16] Therefore, testing with anti-A1 lectin is only necessary in patients who show incompatible crossmatches during the anti-human globulin (AHG) phase. If anti-A1 antibodies are clinically significant, A_2 recipients should receive either A_2 or O blood, and A_2B recipients should be transfused with AHG-compatible red cells from group A_2B, B, A_2, or O. [6, 14, and 17]

As our study did not identify any clinically significant anti-A_1 antibodies in either A_2 or A_2B individuals, routine testing for A_1/A_2 subgroups or anti-A_1 antibodies appears to have limited relevance in this population. This aligns with conclusions from other studies suggesting that in resource-limited settings like India, routine A and AB subgrouping is not recommended.[18] After A_1, A_2 is the next most common A subgroup, with approximate prevalence of 80% and 20%, respectively. Other subgroups, such as A_3 and A end, are extremely rare, occurring in approximately 1 in 14,448 and 1 in 43,344 individuals, respectively. A_3 subgroups are typically identified serologically as large...

A3 subgroups are serologically identified by the presence of large agglutinates resembling a cluster of grapes, seen against a background of numerous unagglutinated cells—a pattern known as mixed field appearance. [2] In our study, we did not observe any A subgroups exhibiting this mixed field pattern, indicating the rarity of such subgroups in our population.

Several studies have reported successful kidney transplants from A_2 blood donors to recipients with blood groups O and B. Therefore, subtyping of blood group A can play a crucial role in optimizing the use of A_2 donors for renal transplantation in patients with O and B blood types. [19] Additionally, subtyping is valuable in managing platelet inventories, as platelets from A_2 and A_2B donors can be safely transfused to O and B group recipients, respectively, with favourable post-transfusion recovery and corrected count increments. [20]

However, it is important to perform subtyping of group A in specific critical situations—for instance, in cases involving weak subgroups of A that are negative for anti-A1 lectin, or in incompatible solid organ transplants. [21] There have been reports of hyper acute rejection of A subgroup renal grafts in O group recipients, highlighting the importance of accurate subtyping in such contexts. [22]

CONCLUSION

To date, no investigations from Southern Indian state of Karnataka have explored the prevalence of A_2 and A_2B subgroups and the presence of anti-A_1 antibodies in both donor and patient populations. Our study indicates that routine screening for anti-A_1 antibodies in individuals with A_2 or A_2B subgroups is a good practice. However, in transplantation settings, it remains mandatory to subtype A and AB blood groups and specifically check for anti-A_1 antibodies. [3]

As it is already standard practice, ABO and Rh blood typing—with subgrouping—should be performed in every case to minimize transfusion-related complications. Additional studies across different regions of the state are needed to better understand the distribution of ABO, Rh blood groups, and their subgroups. This information will also help blood banks anticipate demand for specific blood types and subtypes. [4]

Blood Group	Sub Group	Patients,n(%)	Donor,n(%)	Total,n(%)
A	**A_1** **A_2**	**800(93.8)** **50(6.2)**	**2270(98.7)** **30(1.3)**	**3070(97.4)** **80(2.6)**
AB	**A_1B** **A_2B**	**140(92.9)** **10 (7.1)**	**680(97.1)** **20(2.9)**	**820(96.4)** **30(3.6)**

Table 1: Prevalence of A_2 and A_2B subgroups among A and AB blood groups (*n*=4000, A=3150, AB=850).A total of 4000 blood samples from individuals with A and AB blood groups—including both patients and donors—were analysed.

Among the A group samples, 3070 were classified as A_1 and 380 as A_2. In the AB group, 820 were A_1B and 30 were A_2B.

Table 2: Prevalence of anti-A_1 antibody in A_2 and A_2B subgroups: The anti-A_1 antibodies Were considered clinically insignificant as they did not exhibit agglutination at 37°C.

	A_2 sub groups (n=37)		A_2b Sub groups (n=34)	
	Donors	Patients	Donors	Patients
Presence of antiA_1 antibody	Nil	Nil	Nil	Nil

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