

Frequency of A₂ and A₂B Subgroups among Individuals with A and AB Blood Groups at a Multispeciality Centre in South Canara

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Abstract: ***Background:** The ABO blood group system is one of the most clinically significant blood group systems in transfusion medicine and comprises four major phenotypes: A, B, AB, and O. Blood group A is primarily divided into A₁ and A₂ subgroups, with A₁ being the more common subgroup. Individuals with A₂ or A₂B subgroups may occasionally develop anti-A₁ antibodies. Anti-A₁ is generally considered clinically insignificant when reactive only at lower temperatures; however, antibodies reactive at or near 37°C may be clinically significant and may cause haemolytic transfusion reactions. Identification of A subgroups is therefore important for safe transfusion practice. **Objectives:** To determine the frequency of A₂ and A₂B subgroups among individuals with A and AB blood groups. **Methods:** A cross-sectional study was conducted among patients attending Mangala Hospital and Mangala Kidney Foundation, Mangaluru, Karnataka, India, from January 2024 to December 2024. ABO and RhD blood grouping data of 450 patients were analysed. Individuals belonging to blood groups A and AB were further evaluated for A₁, A₂, A₁B, and A₂B subgroups according to the laboratory protocol. **Results:** Among the 450 individuals studied, 183 (40.7%) were blood group O, 144 (32.0%) were blood group A, 90 (20.0%) were blood group B, and 33 (7.3%) were blood group AB. Regarding RhD status, 427 (94.9%) individuals were RhD positive and 23 (5.1%) were RhD negative. Thus, 177 (39.3%) individuals belonged to the A and AB blood groups. Among the 144 individuals with blood group A, 140 (97.2%) were A₁ RhD positive, 1 (0.7%) was A₂ RhD positive, and 3 (2.1%) were A₁-negative, no A₂-negative individual was identified. Overall, one individual was identified as A₂-positive, corresponding to a frequency of 0.7% among individuals with blood group A. Among the 33 individuals with blood group AB, all 33 (100%) were A₁B RhD positive, while no A₂B subgroup was identified. **Conclusion:** A₂ was an uncommon subgroup in the study population, while A₂B was not identified among the AB blood group individuals studied. Appropriate identification of A subgroups may assist in the investigation of ABO discrepancies and support safe transfusion practices.*

Keywords: ABO blood group, A₂ subgroup; A₂B subgroup, anti-A₁; blood grouping, transfusion medicine, immunohematology, South Canara.

1. Introduction

The ABO blood group system is the most important and clinically significant blood group system in transfusion medicine. Karl Landsteiner discovered the ABO blood group system in 1900, establishing the fundamental principles of ABO compatibility. The ABO system consists of four major blood groups A, B, AB and O based on the presence or absence of A and B antigens on the surface of red blood cells and the corresponding naturally occurring antibodies in plasma. Unlike most other blood group systems, the ABO system is characterized by the predictable presence of antibodies against ABO antigens that are absent from an individual's red blood cells. [1,2]

ABO blood group antigens are carbohydrate structures expressed on glycoproteins and glycolipids present on the red blood cell membrane. These antigens are synthesized through the action of specific glycosyltransferase enzymes encoded by the ABO gene, which is located on the long arm of chromosome 9 (9q34.2). The ABO gene contains seven exons, with exons 6 and 7 encoding most of the catalytic domain responsible for A and B glycosyltransferase activity. [3-5] ABO antigens may also be expressed on various tissues and, in secretors, may be present in body fluids such as saliva.[2]

The blood group A phenotype consists of several subgroups, of which A₁ and A₂ are the most common. A₁ individuals express a greater amount of A antigen on their red blood cells, whereas A₂ individuals have a relatively weaker expression of A antigen. The difference between these subgroups is primarily related to variations in the activity and specificity of the A glycosyltransferase encoded by different ABO alleles.[6] Other A variants, including A₃, A_x, A_{end}, A_m and A_{el}, are considerably less common and may present with varying degrees of weak or mixed-field agglutination during routine serological testing.

Individuals with group AB may similarly be classified as A₁B or A₂B depending on the A subgroup expressed. A₂ and A₂B individuals may occasionally produce anti-A₁ antibodies. Anti-A₁ is usually an IgM antibody and is generally clinically insignificant when it reacts only at lower temperatures. However, in some individuals, anti-A₁ may react at or near 37°C and may become clinically significant during transfusion.[7,8] Therefore, recognition of A₂ and A₂B subgroups is important, particularly when discrepancies are encountered between forward and reverse ABO grouping or when unexpected reactions occur during compatibility testing.

The frequency of A₂ and A₂B subgroups varies considerably among different geographical and ethnic populations. Studies from India have demonstrated regional differences

in the distribution of these subgroups. A study conducted in South India reported A₂ in approximately 1.07% of group A individuals and A₂B in approximately 8.99% of group AB individuals.[9] Similarly, a study from North Karnataka reported A₂ in approximately 1.10% of group A individuals and A₂B in approximately 10.30% of group AB individuals.[10] These variations may be influenced by genetic background, geographical distribution, demographic characteristics, sample size and differences in serological methodology.

Despite the availability of data from different regions of India, limited information is available regarding the frequency of A₂ and A₂B subgroups specifically in the South Canara region. Establishing regional frequencies may be useful for understanding the distribution of ABO subgroups and for assisting transfusion laboratories in the investigation and resolution of ABO discrepancies.

Therefore, the present study was undertaken to determine the frequency of A₂ and A₂B subgroups among individuals with blood groups A and AB attending a multispeciality centre in South Canara and to assess their relevance in routine transfusion practice.

2. Materials and Methods

The present cross-sectional study was conducted among patients attending Mangala Hospital and Mangala Kidney Foundation, Mangaluru, Karnataka, India for a period of one year from January 2024 to December 2024. The data were obtained from patient request forms and laboratory records available in the medical laboratory. The information collected included age, gender, ABO blood group, RhD status, and A subgroup findings. Ethical approval for the study was obtained from the Mangala Institutional Ethics Committee on 12/06/2023 (Ref. No.: MIEC/7.0/115).

The required sample size was calculated using the formula $n = Z^2pq/d^2$, considering an estimated prevalence of 33.44%, a 95% confidence level ($Z = 1.96$), and an allowable absolute error of 5%. The calculated minimum sample size was approximately 342. Considering a 10% allowance for incomplete or missing data, the required sample size was approximately 376. A total of 450 patients were included in the study using a convenience sampling technique.

The blood samples were tested for ABO and RhD blood grouping by the slide agglutination method using appropriate antisera. The patient's blood sample was tested separately with anti-A and anti-B antisera, and the presence or absence of visible agglutination was observed and recorded. RhD grouping was performed using anti-D reagent by the same agglutination method. Based on the observed agglutination reactions, the ABO and RhD blood groups were determined.

Individuals identified as having blood group A and AB, were further evaluated for A subgroup status using anti-A₁ lectin according to the laboratory protocol. A positive reaction with anti-A₁ lectin was recorded as A₁, whereas absence of reaction was recorded as A₂. The subgroup findings were documented as A₁-positive, A₂-positive, A₁-

negative and A₂-negative according to the study laboratory protocol. Individuals with blood group AB were similarly assessed and classified as A₁B or A₂B according to the laboratory protocol.

The collected data were entered, tabulated and analyzed using descriptive statistical methods. Categorical variables were expressed as frequencies and percentages.

3. Results

A total of 450 individuals were included in the study during the one-year study period. Among the study participants, blood group O was the most frequently observed blood group, comprising 183 (40.7%) individuals, followed by blood group A with 144 (32.0%), blood group B with 90 (20.0%), and blood group AB with 33 (7.3%) individuals. Thus, blood group O was the predominant ABO blood group, whereas AB was the least frequently observed blood group.

Table 1: Distribution of ABO blood groups among the study population

ABO blood group	Frequency (n)	Percentage (%)
O	183	40.7
A	144	32
B	90	20
AB	33	7.3
Total	450	100

Regarding RhD status, 427 (94.9%) individuals were RhD positive and 23 (5.1%) were RhD negative. Among the AB group, all 33 individuals were RhD positive. Of the 144 individuals with blood group A, 141 (97.9%) were RhD positive and 3 (2.1%) were RhD negative. Among blood group O individuals, 173 (94.5%) were RhD positive and 10 (5.5%) were RhD negative. In the B blood group, 80 (88.9%) were RhD positive and 10 (11.1%) were RhD negative.

Table 2: Distribution of ABO blood groups according to RhD status

ABO blood group	RhD-positive, n (%)	RhD-negative, n (%)	Total, n (%)
O	173 (94.5)	10 (5.5)	183 (100)
A	141 (97.9)	3 (2.1)	144 (100)
B	80 (88.9)	10 (11.1)	90 (100)
AB	33 (100.0)	0 (0.0)	33 (100)
Total	427 (94.9)	23 (5.1)	450 (100)

Among the individuals belonging to blood groups A and AB, subgroup analysis was performed to determine the distribution of A₁, A₂, A₁B, and A₂B subgroups. Among the 144 individuals with blood group A, 140 (97.2%) were A₁-positive, 1 (0.7%) was A₂-positive, 3 (2.1%) were A₁-negative, and no A₂-negative individual was identified. Thus, the frequency of the A₂ subgroup among individuals with blood group A was **0.69% (1/144)**.

Table 4: Distribution of A₁ and A₂ subgroup findings among individuals with blood group A

A subgroup	RhD-positive, n (%)	RhD-negative, n (%)	Total, n (%)
A ₁	140 (97.2)	3 (2.1)	143 (99.3)
A ₂	1 (0.7)	0 (0.0)	1 (0.7)

Total	141 (97.9)	3 (2.1)	144 (100)
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Among the 33 individuals with blood group AB, all 33 (100%) were A₁B-positive, while no A₂B-positive individual was identified. No A₁B-negative or A₂B-negative individuals were observed. Therefore, the frequency of the A₂B subgroup among individuals with blood group AB was **0% (0/33)**.

Table 5: Distribution of A₁B and A₂B subgroup findings among individuals with blood group AB

AB subgroup	RhD-positive, n (%)	RhD-negative, n (%)	Total, n (%)
A ₁ B	33 (100.0)	0 (0.0)	33 (100)
A ₂ B	0 (0.0)	0 (0.0)	0 (0)
Total	33 (100)	0 (0)	33 (100)

4. Discussion

The present study evaluated the distribution of ABO and RhD blood groups and specifically assessed the occurrence of A₂ and A₂B subgroups among individuals with blood groups A and AB attending a multispeciality centre in South Canara. Among the 450 individuals included in the study, blood group O was the most frequently observed blood group (40.7%), followed by blood groups A (32.0%), B (20.0%), and AB (7.3%). The predominance of blood group O and the relatively lower frequency of AB observed in the present study are consistent with patterns reported in several Indian populations. However, differences in the relative frequencies of ABO groups have been reported between geographical regions, which may be influenced by genetic and demographic characteristics of the study population.

In the present study, RhD-positive individuals constituted 94.9% of the study population, whereas 5.1% were RhD negative. The high proportion of RhD-positive individuals is consistent with the predominance of the RhD-positive phenotype reported among Indian populations. The observed distribution emphasizes the importance of considering both ABO and RhD systems during routine blood-group testing and transfusion practice.

The principal focus of the present study was the identification of A₂ and A₂B subgroups. The A₂ phenotype is the major non-A₁ subgroup of blood group A and is characterized by weaker expression of A antigen compared with A₁. Although A₂ generally occurs at a relatively low frequency among group A individuals, its identification is relevant in transfusion medicine because A₂ and A₂B individuals may develop anti-A₁ antibodies. Such antibodies may occasionally contribute to discrepancies between forward and reverse ABO grouping and may create difficulties during compatibility testing. Therefore, recognition of ABO subgroups is particularly important when unexpected serological reactions are encountered during routine blood grouping.

In the present study, A₂ was identified in 1 of the 144 individuals with blood group A, corresponding to a frequency of **0.69%**. In contrast, A₂B was not detected among the 33 individuals with blood group AB, giving an observed frequency of **0%**. The low frequency of A₂ observed in the present study is consistent with the relatively

uncommon occurrence of this subgroup reported in some Indian populations. The absence of A₂B should, however, be interpreted cautiously because only 33 individuals belonged to the AB blood group. A finding of zero cases in a relatively small sample does not necessarily indicate that the phenotype is absent from the South Canara population.

Previous studies conducted in Indian populations have reported variation in the frequency of A₂ and A₂B subgroups. Shastry and Bhat, in a study from South India, reported an A₂ frequency of approximately 1.07% among group A individuals and an A₂B frequency of approximately 8.99% among group AB individuals.[9] Similarly, Giryani et al., in a study from North Karnataka, reported an A₂ frequency of approximately 1.10% among group A individuals and an A₂B frequency of approximately 10.30% among group AB individuals.[10]

The A₂ frequency observed in the present study (0.69%) was lower than the frequencies reported in these two Indian studies. The absence of A₂B in the present study also differed from the higher A₂B frequencies reported previously. Such differences may reflect variations in geographical distribution, population characteristics, sample size, and serological methodology.

The difference in A₂B frequency between the present study and previous studies may partly be explained by the relatively small number of AB individuals included in the present study. Only 33 individuals belonged to blood group AB; therefore, even a small number of A₂B cases would substantially alter the calculated frequency. Consequently, direct comparison of A₂ and A₂B frequencies between studies should take methodological and population-related differences into consideration.

The identification of A₂ and A₂B subgroups has practical significance in immunohematology. ABO discrepancies involving A subgroups may be encountered during routine blood grouping, particularly in cases of weak antigen expression or when anti-A₁ is present in the serum. Appropriate serological investigation, including the use of anti-A₁ lectin where indicated, can assist in resolving such discrepancies. Accurate identification of ABO subgroups is therefore useful for preventing misinterpretation of blood-group results and supporting safe transfusion practices.

The study has certain limitations. It was conducted at a single multispeciality centre and included a relatively limited number of participants, particularly individuals belonging to the AB blood group. The small number of AB individuals limits the precision with which the frequency of A₂B can be estimated. In addition, the study population consisted of individuals attending the healthcare centre and may not be representative of the entire South Canara population. Subgroup identification was based on serological testing, and molecular characterization was not available to confirm the subgroup phenotype. Future studies involving larger sample sizes, multiple centres, and, where feasible, molecular or genotypic confirmation would provide more robust estimates of A₂ and A₂B frequencies.

Overall, the findings of the present study contribute to the available regional data on ABO subgroup distribution. A₂ was identified in 0.69% of group A individuals, whereas A₂B was not detected among the group AB individuals studied. These findings emphasize the importance of appropriate ABO subgroup investigation, particularly when discrepancies are encountered during routine blood grouping. Further multicentric studies involving larger populations from South Canara and neighbouring regions are recommended to establish the prevalence and clinical significance of A₂ and A₂B subgroups more accurately.

5. Conclusion

A₂ and A₂B are relatively uncommon ABO subgroups with important implications in transfusion medicine. In the present study, A₂ was identified in **0.69%** of individuals with blood group A, whereas A₂B was not detected among individuals with blood group AB. Although these subgroups are uncommon, their identification is important because ABO subgroup-related serological discrepancies may complicate blood-group interpretation and compatibility testing. Therefore, appropriate investigation of A and AB subgroups, particularly in cases of unexpected serological reactions or ABO discrepancies, can contribute to safer and more effective transfusion practices and help prevent avoidable transfusion-related complications.

6. Limitations

The study was conducted at a single multispeciality centre and included a relatively small number of participants, particularly those belonging to the AB blood group. The small sample size may limit the precision of estimating the frequency of A₂B and may restrict the generalizability of the findings to the wider population. Further studies involving larger sample sizes, multiple centres, and a longer study period are recommended to obtain more reliable estimates of A₂ and A₂B subgroup frequencies and to better understand their clinical and transfusion-related significance.

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