A2 Subgroups of Blood Group ‘A’ and ‘AB’ in Tertiary Care Hospital: A Reflection of their Prevalence in Trivandrum

Saritha S, Dr. Vijayalekshmi Kuttath

Abstract: Introduction: The two primary subgroups of A antigen are A1 and A2. About 0.4% of A2 subgroups and 25% of A2B have anti-A1 antibody which agglutinates A1, but not A2 cells. Materials and Method: In this study conducted in a major blood bank, group-wise analysis of 750 donors over three months, with special emphasis on the percentage of A2 and A2B individuals was done. Also, identification of A2 and A2B subgroup was done in patients. The chance of occurrence group A2 & A2B patients transfused with A1 group blood were framed and the percentage of them showing positive ICT were identified. This was done in an attempt to predict the possibility of these A2 subgroups being produced immune anti-A1 antibody to the transfused A1 and A2B red cells. Results: Out of the 632A group donors, A1 antigen were present in 574 (90.8%) and A2 antigen was present in 58 (9.17%) donors. In AB group, 104 were A2B and 14 were A2A2B subgroup was found in 9.17% of A group and 13.46% of A2B. Out of 86A & 14AB group blood patients, 6A1 and 2 A2B patients were found and ICT on these patients revealed that 5 A2 and 1 A2B patient tested positive for irregular antibodies. Conclusion: Routine detection of A2 subgroup will contribute significantly to the prevention of hemolytic reaction by a natural/acquired anti-A1 antibody and will also prevent the mistaken transfusion of A2 subgroup blood to an O recipient.

Keywords: Antibody, antigen, blood groups, donors, transfusion

1. Introduction

The identification of the ABO blood system by Karl Landsteiner marked the commencement of safe blood transfusion system. The ABO blood profile, so far, has been considered as the most vital in transfusion safety. The ABO blood grouping consists of four structural molecules, i.e., A, B, AB, and O. The A antigen has two major subgroups as A1 and A2, depending on which A and AB blood groups have been classified. Individuals with A antigen in blood, 20% have A1 subgroup and 80% have A2 subgroup. Similarly, from individuals with AB antigen in blood, 10.3% belong to A2B and 89.7% belong to the A1B subgroup. The A2 subgroup makes up 1% of those encountered in the laboratory and therefore are mainly of academic interest.

A1 is a very potent gene that creates from 810,000 to 1,170,000 antigen sites on the adult RBC, whereas inheritance of an A2 gene results in the production of only 240 000 to 290000 antigen sites on the adult A2 RBC. A1 and A2 are differentiated based on an antibody, that is, anti-A1 in the serum. Approximately 0.4% of A2 subgroups and 25% of the A2B subgroup possess anti-A1 antibody. Generally, this antibody reacts below 37°C (body temperature) and is simply a medical nuisance causing discrepancies in ABO testing and incompatibilities in crossmatches with A1 or A2B cells. However, this anti-A1 antibody, when active at 37°C, though rare, destroys A1 cells, leading to transfusion reactions, which has been documented. Thus, the objective of the study is to determine the percentage of A2 and A2B subgroups in the studied population (donors and patients) and to look for the presence of anti-A1 in A2 & A2B donors. The study also aimed to assess A2 subgroup patients with irregular antibodies for safe transfusion practices.

2. Materials and Method

The present study was executed using blood samples of donors and patients belonging to blood group A and AB obtained from the Department of Blood Bank in a tertiary care hospital in Trivandrum. The period of the study was three months (May to July) and data were collected taking due permission from the blood bank. The ethical consideration for the study was obtained from the Institutional Ethical Committee. The gender and identity of the donors were not disclosed in any form throughout the study.

2.1 Recognition of A2 and A2B Subgroup and anti-A1 in A2 and A2B

To determine the percentage of A2 and A2B subgroups in donors, 750 samples of blood group A and AB of donors were collected. The subgroups were tested using anti-A protein. Whenever the agglutination was +4 with anti-A antibody but negative with anti-A1 antibody, the sample was considered to be A2 subgroup for blood groups A and AB (Table 1).

Table 1: Testing for A2 and A2B subgroups

<table>
<thead>
<tr>
<th>Reaction of red cells with antiserum</th>
<th>Reaction of serum with cells</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>+4</td>
<td>-</td>
<td>A2*</td>
</tr>
<tr>
<td>+4</td>
<td>-4</td>
<td>-</td>
</tr>
<tr>
<td>+4</td>
<td>+4</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>+4</td>
<td>-</td>
</tr>
</tbody>
</table>

H = Hemolysis; - = No agglutination; *Occasionally A2 and A2B serum contains anti-A1 and thus gives reaction with pooled A cells; + to +4 = agglutination of increasing strength.

The presence of anti-A1 in A2 and A2B subgroups were determined by the tube method. Forward and backward grouping was done using antibodies and the collective A, B and O cells. 1 volume each of anti-A1 protein reagent was taken into three clean test tubes and an equal amount of 2% saline culture of the donor’s/patient’s red blood cells was
added to the test tubes. To the second tube, an equal volume of known A cells was added and to the third one, an equal volume of known Ac cells (A1 and A2 cells as positive and negative controls should always be included) was added. The contents of each tube were mixed mildly by brisk shaking and incubated at room temperature for 30-60 min. After incubation, the tubes were centrifuged at 1000 rpm (revolutions/min) for 1 min followed by examination of agglutination. The forward typing result was confirmed by verifying the presence of antigens in the blood and the backward typing result was confirmed by the existence of antibodies in the serum sample.

2.2 Indirect Coomb Test (ICT) for Identification of Irregular Antibodies in Patients

To identify irregular antibodies in A2 and A2B recipients, 100 multi-transfused patients with 86A & 14 AB blood group were selected. The chance of occurrence group A2 & A2B patients, transfused with A1 group blood were determined using protein and the percentage of them showing positive ICT were determined by indirect comb test to predict the possibility of these A2 subgroups being produced immune anti-A1 antibody to the transfused A1 and A2B cells. Serum of A2 and A2B groups of patients were taken in separate test tubes and a drop of culture of O positive cells (5%) was added to each test tube and mixed mildly followed by incubation at 37°C for 30-45 min. This was followed by centrifugation at 1000 rpm for 1 min to separate the content in the test tubes and observed for clumping. Washing of the cells was done thrice with warm saline and a drop of anti-human globulin (AHG) reagent was added to the last cell sediment for observation under the microscope. Agglutination was interpreted as ICT positive.

2.3 Statistical Methodology

The occurrence of subgroups among the blood groups were described in percentages. The significance of the association between the observed frequency of subgroups with the frequency of blood groups and presence of irregular antibody was analyzed using chi-square statistics.

3. Results

3.1 Recognition of A2 and A2B Subgroup and anti-A1 in A2 and A2 B

ABO grouping was analyzed for 750 donors over three months. Of these, 632 (84.3%) belonged to group A and 118 (15.7%) belonged to group AB. From the 632 donors of the A group, A1 antigen was present in 574 (90.8%) donors and A2 antigen was present in 58 (9.2%) donors (Table 2). In the case of the AB group, from the 118 donors, A1B antigen was present in 104 (88.1%) donors and A2B antigen was present in 14 (11.9%) donors. From the total study population, A antigen was found in all donors (A and AB blood groups). A significant association was found between the observed frequency of the sub-groups and frequency of the blood groups at p < 0.05 (Table 2). Out of this, A1 antigen was found in 72 (9.2% of A group and 11.9% of AB group) donors. In this study, it was found that A2 as A2B among AB blood group donors was in higher numbers than A1 among A blood group donors, being statistically significant. From a total of 72 A2 donors, 2 donors had anti-A1 among 14 A2B donors and 1 donor had anti-A1 among 58 A2 donors (Figure 1).

<table>
<thead>
<tr>
<th>Blood group</th>
<th>Subgroup</th>
<th>A</th>
<th>A2</th>
<th>A1</th>
<th>A2B</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Count</td>
<td>574</td>
<td>0</td>
<td>58</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>% within blood group</td>
<td>90.8%</td>
<td>0.0%</td>
<td>9.2%</td>
<td>0.0%</td>
</tr>
<tr>
<td>AB</td>
<td>Count</td>
<td>0</td>
<td>104</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>% within blood group</td>
<td>0.0%</td>
<td>88.1%</td>
<td>0.0%</td>
<td>11.9%</td>
</tr>
</tbody>
</table>

Chi-Square=750.000; p < 0.05

Figure 1: Presence of anti-A1 among A2 and A2B donors

3.2 Indirect Coomb Test (ICT) for identification of irregular antibodies in patients

Among a total of 100 multi-transfused patients, 86 belonged to A group & 14 belonged to AB group patients. From the 86 patients of the A group, A3 antigen was present in 6 (7%) patients and A1 antigen was present in 80 (93%) patients. In the case of 14 patients of the AB group, A3B antigen was present in 2 (14.3%) patients and A2B antigen was present in 12 (85.7%) patients, the association between frequency of subgroups and blood groups being statistically significant (Table 3). Out of the total study sample (100), A antigen was present in all patients (A and AB blood groups), out of which, A1 was present in 8 patients. In this study, it was found that A2 as A2B donors among A2B blood group patients was present in higher numbers than A1 among A blood group patients, found to be statistically significant.

<table>
<thead>
<tr>
<th>Blood group</th>
<th>Subgroup</th>
<th>A</th>
<th>A2</th>
<th>A1</th>
<th>A2B</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Count</td>
<td>80</td>
<td>0</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>% within blood group</td>
<td>93.0%</td>
<td>0.0%</td>
<td>7.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td>AB</td>
<td>Count</td>
<td>0</td>
<td>12</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>% within blood group</td>
<td>0.0%</td>
<td>85.7%</td>
<td>0.0%</td>
<td>14.3%</td>
</tr>
</tbody>
</table>

Chi-Square=100.000; p < 0.05

The ICT performed on the A2 and A2B patients showed that out of 6 A2 patients, 5 A2 tested positive for irregular antibodies (83.3%) and out of 2 A2B patients, 1 A2B (50%) tested positive for irregular antibodies, being statistically significant (Table 4).
The presence of irregular antibody among A2 and A2B have been graphically represented in Figures 2 and 3.

![Irregular Antibody Among A2](image1)

**Figure 2:** Presence of irregular antibody in A2 subgroup patients

![Irregular Antibody Among A2B](image2)

**Figure 3:** Presence of irregular antibody in A2B subgroup patient

### 4. Discussion

The ABO blood profile system with antigens A, B and O has different phenotypic variants (A1, A2, B, A, B, A2B, and O). The frequency of these variants differs based on different populations. In this blood grouping system, group A constitutes 44.6% of all blood groups with 80% prevalence of A1 and 20% prevalence of A2, and AB constitutes the lowest proportion of all blood groups. In the present study, out of 750 samples of A and AB blood groups, A group was dominant in 84.3% and the AB group was dominant in 8.2% donors, indicating a higher prevalence of A group compared to the AB group. This is similar to a study from Sudan, where A and AB blood antigen prevalence was found to be 76% and 24%, respectively. In another study from South Gujarat, India, the incidence of A group (24.35%) was lesser compared to O and B groups, while the incidence of AB group was only 8.94%. Similarly, in another study from Sikkim, India, the incidence of A group (22.91%) was lesser compared to O and B groups, while the incidence of AB group was only 14.12%.

In the present study, the prevalence of A1 and A2B was 90.8% and 88.1%, and A2 and A2B were 9.17% and 11.7% respectively of the donor samples in the blood bank. In a similar study, the prevalence of A1 and A2B was 98.3% and 89.7%, while the prevalence of A2 and A2B was 0.85% and 1.21% respectively. The present study indicated A1 as the most common subgroup among A group and A2B as the most common among the AB group, indicating A1 and A2B to be still the uncommon subgroups. A similar distribution was found in the Indian population. A similar result was reported in Gwalior, India, where the prevalence of A1 and A2B was 8% and 8.6% respectively. In another study, the prevalence of A1 and A2B was found to be 4.1% and 19.2%. A1 and A2B constitute the major subtypes of ‘A’ blood group. A2 and A2B are rare subgroup individuals, where a small proportion of A2 and a higher proportion of A2B do not recognize A1 antigens as a part of their own RBC and produce specific anti-A1 antibody against A1 cells. Thus, the implication of weaker variants like A2 is important as they may generate a mistyping with AB as B group and A as O group, thereby triggering hemolysis during transfusion.

In the present study, the occurrence of irregular antibody was found to be 14.2% among A2B donors (2 anti-A1 out of 14) and was 1.7% among A2 donors (1 anti-A1 out of 58). This occurrence of anti-A1 among the A2 donors might reflect a past occurrence of blood transfusion, presuming that anti-A1 antibody in their sera could be natural. In a similar study, 21% anti-A1 antibody in A2B donors and 10% anti-A1 antibody in A2 donors was reported. However, if the development of antibody to A1 in A2 individuals occurs, then determining the subgroup for successive transfusion is imperative. It becomes critical at this point to detect a weak subgroup of ‘A’, failing which may result in a patient or donor being mistyped as group AB or B or O.

The present study revealed that the incidence of A1 patients as A2B among the AB group was significantly higher than the A1 patients among A group, indicating an imbalance in A2 and A2B frequencies in A and AB positive patients. This finding corroborates with the other studies. This imbalance might be attributed to the fact that the dominant B
gene might suppress the expression of A1 antigen, leading to the higher expression of A2B in the studied population.

Further, the present study revealed that anti-A1 was exhibited by 83.3% A2 patients (five out of six A2) and 50% of A2B patients (one out of two A2B). Some events can cause unexpected or erroneous serum test result. Patients with immunodeficiency may not produce a measurable amount of anti-A1 and anti-B. On the other hand, abnormally high concentrations of anti-A1 can lead to false-negative results. Anti-A1, which is reactive in vitro at about 30°C but only dubiously active at 37°C, will destroy some of the A1 cells in vivo when a small dose of cells is injected. These antibodies, whose activity is uncertain at 37°C would nearly fail to detect RBC destruction following the transfusion of therapeutic quantities of blood. On the other hand, cases where anti-A1 has been active at 37°C, extensive destruction of A1 cells in vivo has been recorded.

If these people become blood recipients they can have an immediate hemolytic reaction during further transfusion due to a natural/immune anti-A1 antibody. This points to the fact that by failing to do anti-A1 agglutination throughout A & AB blood, we fail to detect many A2 & A2B subgroups and some A2 subgroup gets detected as O. Also mistaken transfusion of an A2 subgroup blood (which did not give agglutination with anti-A1 protein and hence misinterpreted as O) to an O recipient can cause immediate hemolysis in the recipient with unpredictable severity, indicating the significance of A2 subgroup in transfusion and the importance of detecting anti-A1 in blood group testing to differentiate between A1 and A2 individuals from A and AB groups.

The present duration of the study can be prolonged for years to produce an inclusive and more reliable data. The total sample taken for the study should be increased to derive better statistically significant data. The identification of the prevalence of other weak variants of A group can be established in future studies.

5. Conclusions

In conclusion, the A2B subgroup was found to be more prevalent than the A1 subgroup in the studied population. Both the subgroup A2 and A2 reacted with anti-A, however, anti-A1 antibody differentiated them as it does not react with A2 cells. Routine detection of A2 subgroup will contribute significantly to the prevention of hemolytic reaction by a natural/acquired anti-A1 antibody and will also prevent the mistaken transfusion of A2 subgroup blood to an O recipient. The study found that anti-A1 was not present more in donors, but the testing for anti-A1 should be carried out before blood transfusion to prevent any reaction.

References


