has A, B, AB, and O blood groups. A and AB again have been divided into subgroups due to polymorphism in the genes encoding for the ABO blood group system (A and B genes). They differ both qualitatively and quantitatively. A1 red cells have 8.1 - 11.7 x 10^5 antigenic sites on adult RBC, whereas inheritance of an A2 gene results in production of only 2.4 - 2.9 x 10^5 antigenic sites. Qualitative differences are due to presence of anti-A1 in 1-8% of A2 and 25% of A2B individuals.

**Table I: antigen and antibody of subgroup AB**

<table>
<thead>
<tr>
<th>Blood group</th>
<th>Antigen</th>
<th>Antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1 B</td>
<td>A, A1 , B</td>
<td>None</td>
</tr>
<tr>
<td>A2 B</td>
<td>A, B</td>
<td>Occasionally A1 (22 - 26%) (article 3)</td>
</tr>
</tbody>
</table>

Introduction:

In 1900 Karl Landsteiner discovered ABO blood group system on basis of presence of agglutinogen on red cell membrane and agglutinin in serum. The ABH antigens are expressed by addition of terminal monosaccharide immunodominant sugar to precursor oligosaccharide H chain, and they are present in various tissues or cells such as platelets, epithelium, vascular endothelium, intestinal, cervical, urothelial, and mammary epithelial cells. Blood group plays the vital role in blood and blood components transfusion, haematopoietic stem cell transplant as well as organ transplantation. This significance is due to the presence of naturally occurring IgM anti A, anti B or anti AB antibodies which are active at 37°C.

The International Society of Blood Transfusion (ISBT) has recognized 346 blood group antigens, out of which 308 have been designed in 36 blood group systems while 38 are still not assigned to any blood group system.1 ABO and Rh blood group systems are considered as major blood groups. ABO blood group system AB constitutes the lowest proportion of all blood groups. 80% of A subgroup belong to A1 while 20% belong to A2 and individuals with AB antigen in blood 10.3% belong to A2B and 89.7% belong to A1B subgroup.2 Table I shows antigen and antibody present in AB subgroups. In routine testing, red cells of both A1B and A2B subgroup strongly agglutinate with monoclonal anti A reagent. However, A1 and A2 cells can be distinguished from each other by anti-A1 lectin of Dolichos biflorus, which agglutinate A1 red cells but not A2 red cells. Whenever the agglutination was 4+ with anti A antibody but negative with anti-A1 lectin, the sample is considered to be A2 subgroup for blood group A or
AB. Subgroups can result in ABO blood group discrepancy and rarely may lead to haemolytic transfusion reactions. Here we report an incidental case of A2B blood group.

**Case report:**

A 28-year woman came for master health checkup which included ABO and RhD blood grouping. Transfusion medicine department received 3ml EDTA sample. A 3% saline suspension of washed red cells was prepared from sample. Blood grouping was performed with column agglutination technique using Ortho BioVue ABD forward and reverse cassettes (Ortho Clinical Diagnostics, USA). For reverse grouping, in house prepared pooled A cells, B cells and O cells were used. Her forward grouping showed AB and reverse grouping showed B RhD positive (Table II). This discrepancy took our attention to this sample.

### Table II: Blood grouping

<table>
<thead>
<tr>
<th>Method</th>
<th>Forward Grouping</th>
<th>Auto Grouping</th>
<th>Reverse Grouping</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column Agglutination</td>
<td>+ +</td>
<td>Anti</td>
<td>Anti AB</td>
</tr>
<tr>
<td></td>
<td>Not</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>O</td>
<td>Cells Cells Cells</td>
</tr>
<tr>
<td>Tube Method</td>
<td>+ + +</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td>done</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

+ agglutination. - no agglutination

Firstly, the possibilities of technical error and pooled cell suspension related errors were ruled out. Blood group of the patient was repeated by tube method. Both cell and serum grouping were undertaken. Tube method also showed the same result. This interpreted the possibility of A2B blood group. Then anti A1 lectin was used for confirmation with positive and negative control. The content of all test tubes was mixed by gentle shaking and centrifuged at 1000 rpm for 1 minute. Results were seen both macroscopically and microscopically. Patient’s sample showed no agglutination with anti-A1 lectin (Table III). Based on these results, it was typed as A2B subgroup of AB blood group.

### Table III: Results of Anti A1 Lectin

<table>
<thead>
<tr>
<th>Patient sample</th>
<th>Positive control</th>
<th>Negative control</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

Further, her plasma was tested with A1 red cells which confirmed the presence of anti-A1 antibody. The thermal amplitude of anti-A1 antibody was determined by keeping the test tube at 4°C, 22°C and 37°C. The anti-A1 antibody was not clinically significant as it was not reactive at 37°C. Then strength was measured with titration. In semi quantitative method, serum was serially (1:1, 1:2, 1:4...) diluted and the reaction in the last tube showing agglutination was noted as the titre of the antibody. Her antibody titration revealed 32 at 4°C and 16 at 22°C. On further testing with anti H lectin showed 3+ reaction.

Her family history revealed her mother’s blood group is AB positive, father B positive and brother B positive but subgroup was not confirmed to anyone. She had no history of pregnancy or blood transfusion. She was advised to transfuse with group AB RhD positive, strict 37°C IAT cross match compatible blood if needed in future.

**Discussion:**

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. This anti A1 exists as naturally occurring IgM with thermal amplitude of <25°C and is simply a medical nuisance causing discrepancies in ABO grouping and incompatibilities in cross matches but poses no problem in transfusion. Shastry and Bhat found anti A1 in 1.8% of A2 and 3.75% of A2B individuals but none of them were clinically significant.3 However, there are some documented reports where it was shown that this anti A1 antibody could be activated at 37°C and destroys A1 cells leading to transfusion reactions.4-7 Development of

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anti-A1 antibodies after allogeneic SCT and organ transplantation has been reported. Hence, typing with anti-A1 lectin will be required only in patients showing incompatible cross match in AHG phase. From transfusion perspective A2B recipients with clinically significant anti A1 should receive AHG cross-match compatible red cells of group A2B, B, A2 or O.10 The higher prevalence of anti-A1 in A2B subgroup than A2 could be due to the R101 allele (41% in A2B vs 1% in A2).11 A2 phenotype reflects inefficient conversion of H antigen to A antigen for which A2 red cells have increased reactivity with the H lectin of Ulex europaeus.

Frequency of A2 varies in different part of the world. In Indian population frequency of A2 is 0.8% to 3.0% and A2B is 0.6-1.4%.12,13 Giriyan et al found A2 and A2B in 0.8% and 2.5% cases respectively.2 Another pilot study showed A2 and A2B was 4.1% and 19.2%.14 A study in Trivandrum found 13.46% A2B in AB blood group.15 In Bangladesh, the frequency of A2B among AB was found 7%.16 Most of the individuals with a rare blood group are incidentally identified during a routine testing. Our case was also identified incidentally. The patient was counselled and advised to receive AHG cross match blood if needed. A molecular characterization would have been useful in this regard but could not be performed. The establishment of a national registry of rare blood groups donors would aid in creating awareness of their existence and would help to save lives at the time of need.

**Conclusion:**
The case report highlights the need to be aware of such uncommon and rare blood groups and using anti A1 lectin as a standardized protocol to prevent blood group incompatibility.

**References:**


