A CASE OF BLOOD GROUP A₂B WITH ANTI A₁ ANTIBODY REACTIVE AT 37°C

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Abstract:
Landsteiner discovered ABO blood group system. It is most important for transfusion medicine. Blood group A has several subtypes and most important are A₁ and A₂, upon which further groups of A and AB have been classified as A₁, A₂ and A₁B, A₂B. Of individuals with A antigen, approximately 20% belong to A₂ while rest 80% belong to A₁. Anti-A₁ Lectin, a cold agglutinin which destroys A₁ cells is clinically significant when they react at 37°C, causing transfusion reactions.

Key words: ABO blood group, subtypes A₁ and A₂, Anti-A₁ Lectin.

Introduction:
ABO blood group was the first blood grouping system discovered by Landsteiner. It includes different genotypes and phenotypes of A, B and O antigens. Two principal subgroup of blood group A are A₁ and A₂. Subgroups of A can result in discrepancy in ABO blood grouping. But haemolytic transfusion reactions are.¹¹A₂B individuals can have anti A₁ antibodies which reacts at temperature below 25°C and do not produce problem in transfusion.¹¹ Reactivity of anti-A₁ at 37 °C can leads to haemolytic transfusion reaction. As ABO discrepancy leads to haemolytic transfusion reaction, hence it is necessary to include anti A₁ lectin in blood grouping Standard Operative Procedure (SOP)

As the patient required immediate transfusion, he was advised to transfuse antigen negative blood ie, transfused with A₂B, B or O group blood unit.

Case report:
A 25 year old female, known chronic kidney disease was admitted in NIKDU,Dhaka with severe anaemia. Her haemoglobin dropped to 4.5 gm/ dl, serum creatinine was 6.2 gm/dl. Urgent blood demand was placed and blood specimen for grouping and cross-match was collected.

Initial forward and reverse grouping result revealed AB and B and Rh typing revealed D positive. And compatibility test with three units of AB, RhD positive blood at room temperature and at 37°C were incompatible. Compatibility test by Indirect Coombs

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Test (ICT) at 37°C with anti human globulin (AHG) was also incompatible. The blood group of the patient was repeated with ‘washed RBC’ by tube method. Both forward and reverse grouping were done and same result noted. Results were confirmed both macroscopically and microscopically. As there was no anti A1 lectin in our centre, the sample sent to reference laboratory of BSMMU, Dhaka. The blood group of the patient was repeated with ‘washed RBC’ by tube method. Both forward and reverse grouping were undertaken. For forward grouping anti A1 lectin was also taken. Blood group reaction was confirmed macroscopically and microscopically (Table:1). For reverse grouping A1 cells, O cells and A2 cells were taken. Indirect Coombs test at 37°C revealed agglutination due to patients anti A1. After evaluating the all results the blood group of patient was confirmed as A2B with anti A1 antibodies and RhD positive. The thermal amplitude of anti-A1 antibodies was determined by keeping the test tubes at 4°C, 22°C, and 37°C. Next step was the compatibility test for transfusion of safe blood. Patient was asked for family screening, but they failed. Due to non-availability of A2B group blood unit, compatibility test with two units each of O Rh positive and B Rh positive was undertaken. Blood group O units had minor match problems as expected due to donor anti B, however, both B group blood units were found compatible. A decision to transfuse B or O units was taken. In total 4, 2 and 2 units of A1B, O and B Rh positive group blood units respectively were subjected for compatibility test. Result of compatibility test is presented in (Table:2). B group units had two types of compatibility results due to absence and presence of anti A2 which was confirmed by using A1 and A2 cells.

**Table I**

*Results of blood grouping of patient*

<table>
<thead>
<tr>
<th>Test reagents</th>
<th>Anti A</th>
<th>Anti A1</th>
<th>Anti B</th>
<th>Anti AB</th>
<th>Anti D</th>
<th>A1 Cells</th>
<th>A2 Cells</th>
<th>B Cells</th>
<th>O Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Result</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

. + agglutination. – no agglutination

**Table II**

*Results of compatibility tests with several blood donor units*

<table>
<thead>
<tr>
<th>Donor Units</th>
<th>Major Compatibility Test</th>
<th>Minor Compatibility Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline- RT ICT-37°C</td>
<td>Saline-RT ICT-37°C</td>
</tr>
<tr>
<td>A1B RhD+</td>
<td>+  +</td>
<td>N  N</td>
</tr>
<tr>
<td>O Rh D+</td>
<td>N  N</td>
<td>+  +</td>
</tr>
<tr>
<td>B RhD+</td>
<td>N  N</td>
<td>N  N</td>
</tr>
</tbody>
</table>

ICT- Indirect Coomb’s Test. RT- Room temperature, N-Negative/Not agglutinated (compatible)

**Table III**

*Antigen and antibody of ABO system*

<table>
<thead>
<tr>
<th>Blood Group</th>
<th>Antigen</th>
<th>Antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>B</td>
<td>B</td>
<td>A</td>
</tr>
<tr>
<td>AB</td>
<td>AB</td>
<td>No A or B</td>
</tr>
<tr>
<td>O</td>
<td>No A or B</td>
<td>A, B</td>
</tr>
</tbody>
</table>

**Table IV**

*Antigen and antibody of subgroup A1*

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Antigen</th>
<th>Antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>A1</td>
<td>Anti B</td>
</tr>
<tr>
<td>A2</td>
<td>A2</td>
<td>Anti A1</td>
</tr>
<tr>
<td>A1B</td>
<td>A1B</td>
<td>None</td>
</tr>
<tr>
<td>A2B</td>
<td>A2B</td>
<td>Anti A1</td>
</tr>
</tbody>
</table>
Discussion:
Landsteiner’s ABO system of blood groups is most important for transfusion medicine. Major ABO blood group antigens are A, B, AB and O. A\(_2\) and A\(_2\)B are rare subtypes of ABO blood group system. Other less prevalent subtypes of A include A\(_3\), A\(_x\), A\(_{end}\), A\(_y\) and A\(_{sl}\). Differences between A\(_1\) and A\(_2\) are quantitative as well as qualitative. Qualitative difference of A\(_1\) and A\(_2\) lies in their chemical structures. Individuals with A phenotype express A\(_a\), A\(_b\), A\(_c\) and A\(_d\) determinants while A\(_2\) have only A\(_a\) and A\(_b\) antigenic determinants. Absence of A\(_c\) and A\(_d\) is assumed to be a cause of development of anti-A\(_1\) in A\(_2\) and A\(_2\)B individuals.\(^1\) Usually anti-A\(_1\) exist as naturally occurring IgM with a thermal amplitude of less than 25°C. However, the development of anti-A\(_1\) reacting at 37°C have also been reported in the literature.\(^4,5\) Anti-A\(_1\) is important as it is one of the causes of ABO discrepancies, it can develop hemolytic transfusion reaction and its clinical manifestations has also been reported in hemopoietic stem cell and organ transplantation.\(^6,7\) About 0.4% of A\(_2\) and 25% of A\(_2\)B have anti A\(_1\) antibodies.\(^8\)

A\(_1\) and A\(_2\) are distinguished by the reactivity of lectin i.e., anti-A\(_1\) which occurs as a cold agglutinin and exclusively agglutinates A\(_1\) cells. About 0.4% A\(_2\) and 25% of A\(_2\)B subgroups possess anti-A\(_1\). These antibodies become clinically significant if they react at 37°C destroying A\(_1\) cells.\(^9\)

Approximately, 20% of individuals having A antigen in blood belong to A\(_2\) and thus, forming either A\(_2\) or A\(_2\)B subgroups while rest 80% belong to A\(_1\), so as to form either A\(_1\) or A\(_2\)B subgroups.\(^10,11\) Subgroups of A antigen weaker than A\(_2\) are not frequent.\(^9\)

For blood groups positive for A antigen, i.e., group A and AB, further testing with anti-A\(_1\) lectin was conducted.\(^12\) The individuals were hence, classified under sub-blood groups containing A\(_1\) or A\(_2\).

In a study done on the Muslim population of UP by Hussain R et al., the prevalence of A1 and A1B was 26.52% and 19.34% and A2 and A2B was 2.90% and 1.24% respectively.\(^13\) Their study was similar to a study done by Ara G et al., in which prevalence of A1, A1B, A2 and A2B was 24.64%, 20.21% 3.97% and 1.60% respectively.\(^14\) A study by Chaitanya Kumar IS et al., concluded that prevalence of A2 and A2B is 0.85% and 1.21% respectively.\(^15\) In a study done by Sharma D et al., in which A2 and A2B were found to be 8% and 8.6% respectively.\(^16\)

One study has reported a case of IgG anti-A\(_1\).\(^11\) Similarly, two other studies have shown hemolytic transfusion reaction due to anti-A\(_1\).\(^17,18\) Development of anti-A\(_1\) antibodies after allogeneic stem cell transplantation and organ transplantation has also been reported.\(^19\)

It has been reported that individuals with A\(_2\)B phenotype are more prone to develop anti-A\(_1\) as compared to A\(_2\). This could be explained on the basis of two observations. Firstly, individuals with A\(_2\)B have a smaller number of A antigens in comparison to A\(_2\). Secondly, A\(_2\)B individuals possess *R101 allele more commonly than A\(_2\) individuals (41% vs 1%) leading to the high frequency of A\(_2\)B phenotype.\(^20\)

In our case anti-A\(_1\) is reactive at 37°C and transfusion with A\(_2\)B blood group unit is ideal but it is not available. Transfusion of O group red cell is also recommended in these recipients. As blood component facility is available at NIKDU, we advised to transfuseO group Red Cell Concentrate units as we anticipated anti B in plasma of O whole blood units leading to haemolytic transfusion reactions considering large volume transfusion requirements. The other choice left is transfusion of B group units. In majority ofB positive cases primary anti A is anti A\(_1\) however primary anti A\(_2\) can be encountered. It is well known that anti-A in B blood group donors generally have primary anti A\(_1\) and occasionally anti A\(_2\). In management of this case, the importance of blood grouping and AHG phase in compatibility test has been highlighted.\(^21\)

Published cases that have reported clinically significant anti-A\(_1\) antibody are in patients who had undergone cardiac surgery using cold
cardioplegia. Cases of anti-A1 reactive at 37°C leading to transfusion reactions are rare.

From a transfusion perspective, individuals with A2 and A2B should be transfused with identical blood types. However, due to its rarity especially A2B, special attention should be given if identical blood type is not available and the patient needs transfusion of Red Cell Concentrate. These individuals can be transfused with O or B group Red Cell Concentrate considering it the next compatible group.

CONCLUSION:
As the prevalence of anti-A1 in A2 and A2B is rare, incorporating them into the ABO grouping system, to rule out the possibility of its wide range of thermal reactivity, can limit these minor but dangerous transfusion incompatibilities. Any discrepancy in these individuals should be resolved before blood/component transfusion.

Conflict of Interest:
The author stated that there is no conflict of interest in this study

Funding:
No specific funding was received for this study.

Ethical consideration:
The study was conducted after approval from the ethical review committee. The confidentiality and anonymity of the study participants were maintained.

References:


