Original Article

Subgroups of A in Thai Blood Donors

Pimol Chiewsilp, Chayaporn Pinyopornpanich, Sarika Makechay, Jintana Tubrod, Udom Tingtoy
and Sineenart Oota
National Blood Centre, Thai Red Cross Society

Abstract:
The frequency of subgroups of A was found to be varied among different populations. The occurrence of anti-A1 in some cases may lead to the problem in blood transfusion, if they were clinically significant.

Objective: This study aimed to reveal the frequency of A subgroups in Thai blood donors. The occurrence and reactivity pattern of anti-A1 in A\textsubscript{2}, A\textsubscript{2}B and other A subgroup individuals were also studied. Materials and Methods: A total of 13,028 group A and 4,563 group AB were identified during July 10, 2017 to August 5, 2017. The techniques used in this study included standard tube technique for agglutination and indirect antiglobulin test.

Results: Among group A, the frequencies of A\textsubscript{1}, A\textsubscript{2} and A\textsubscript{3} were 13,000 (99.78%), 23 (0.18%) and 5 (0.04%), respectively. For group AB, the frequencies of A\textsubscript{1}B, A\textsubscript{2}B and A\textsubscript{3}B were 4,483 (98.25%), 59 (1.29%) and 21 (0.46%), respectively. Approximately, 21.30%, 4.63%, 54.63% and 19.44% of total 108 A and AB subgroups were A\textsubscript{2}, A\textsubscript{3}, A\textsubscript{2}B and A\textsubscript{3}B, respectively. Anti-A1 detected at 4°C was higher in number than at room temperature for A\textsubscript{2} and A\textsubscript{2}B. The most common reactivity pattern that showed discrepancy in ABO typing was for A\textsubscript{2}B with weak anti-A1. All of anti-A1 found were of no clinical significance.

Conclusion: The low frequency of A subgroup distribution in group A and group AB and the imbalance in A\textsubscript{2} and A\textsubscript{2}B phenotype frequencies of ABO group in Thai blood donors were observed. In addition, anti-A1 in all subgroups of A in this study showed no clinical significance.

Keywords: ● Subgroups of A ● Anti-A1 ● Non-A1 ● Imbalance of A\textsubscript{2} and A\textsubscript{2}B phenotypes

นิพนธ์ต้นฉบับ
หมู่ย่อยของหมู่โลหิต A ในผู้บริจาคโลหิตไทย

พิมล เชี่ยวศิลป์ ชญาภรณ์ ภิญโญพรพาณิชย์ สาริกา เมฆฉาย จินตนา ทับรอด อุดม ติ่งต้อย และ สิณีนาฏ อุทา
ศูนย์บริการโลหิตแห่งชาติ สภากาชาดไทย

บทคัดย่อ
ความถี่ของหมู่เลือดย่อย A ของหมู่ A และ AB มีความหลากหลายในประชากรต่างๆ และที่สำคัญคือ การพบว่า ชีวินของหมู่เลือดย่อย OAB มีความสำคัญทางคลินิก อาจทำให้เกิดปัญหาในการรับโลหิตได้ วัตถุประสงค์เพื่อศึกษาความถี่ของหมู่ย่อยของ A ในผู้บริจาคโลหิตไทย รวมถึงการปริมาณของ anti-A1 ในคนหมู่ย่อย A, A, B และหมู่ย่อยชนิดอื่นๆ วิสูตรและวิธีการ ทำการศึกษาระหว่างวันที่ 10 กรกฎาคม ถึง 5 สิงหาคม พ.ศ. 2560 ได้ตรวจคัดตัวอย่างเลือดหมู่ A จำนวน 13,028 ตัวอย่าง และหมู่ AB จำนวน 4,563 ตัวอย่าง โดยทดสอบปฏิสนธิกับยีสิสท์และ indirect antiglobulin test ตัววิสูตรทดสอบผล การศึกษา ในกลุ่มหมู่ A ความถี่ของ A, A, และ A เท่ากับ 13,000 (99.78%), 23 (0.18%) และ 5 (0.04%) ตามลำดับ ส่วนหมู่ AB ความถี่ของ A, A, และ A, B เท่ากับ 4,483 (98.25%), 59 (1.29%) และ 21 (0.46%) ตามลำดับ anti-A1 ที่ตรวจได้ที่อุณหภูมิ 4°C มีจำนวนเพิ่มขึ้นจากที่ตรวจได้ที่อุณหภูมิห้อง สำหรับ A, และ A, B สำหรับปฏิสนธิกับยีสิสท์ที่ทำให้มีความไม่สอดคล้องระหว่างเซลล์และซีรัมของหมู่เลือด ABO คือ กลุ่ม A, A, และ A, B ที่มี anti-A1 สำหรับ anti-A1 ที่พบทั้งหมดในการศึกษา นี้ทำปฏิสนธิกับยีสิสท์และเป็นชนิดที่ทำปฏิสนธิกับยีสิสท์ได้ในอุณหภูมิต่ำ ซึ่งนับได้ว่าไม่มีความสำคัญทางคลินิก สรุป ความถี่ของหมู่ย่อยของ A ในหมู่ A และ AB พบได้ไม่น้อยมาก และพบความไม่สมดุลของ A, และ A, B ของหมู่เลือด ABO นอกจากนี้ยังพบว่า anti-A1 ที่ตรวจได้ในหมู่ย่อยทั้งหมดของ A ในกลุ่ม A ไม่มีความสำคัญทางคลินิก

คำสำคัญ : ● Subgroups of A ● Anti-A1 ● Non-A1 ● Imbalance of A, and A, B phenotypes

J Hematol Transfus Med  Vol. 27  No. 4  October-December 2017
Introduction

The distribution of ABO blood group was observed to be varied among ethnic groups.\textsuperscript{1,3} Besides the variation in the distribution of A subgroup was also recognized.\textsuperscript{4,5} A\textsubscript{1} is differentiated from A\textsubscript{2} on the basis of agglutination of A\textsubscript{1} cells but not A\textsubscript{2} cells with lectin anti-A1 or monoclonal anti-A1. Other subgroups of A can be defined by observing their weak reactivity with anti-A which are A\textsubscript{2}, A\textsubscript{end} and A\textsubscript{X}, while A\textsubscript{m}, A\textsubscript{y} and A\textsubscript{el} are not.\textsuperscript{6} It can be further serologically differentiated by additional techniques,\textsuperscript{7} such as agglutination strength of cells with anti-H, detection of anti-A1 in reverse grouping, adsorption elution technique with polyclonal anti-A from group B and group O individuals, and detection of H and/or A substances in saliva.\textsuperscript{7}

The individuals with subgroup of A, as A\textsubscript{2}, A\textsubscript{2}B and others so called non-A\textsubscript{1} may possess anti-A1 in their sera. Although it usually demonstrates a low temperature amplitude and is of no clinical significance,\textsuperscript{8} However, anti-A1 had been reported as the cause of acute hemolytic transfusion reaction\textsuperscript{9} as well as severe delayed hemolytic transfusion reaction.\textsuperscript{10} Then it is important to study the nature of this antibody in addition to unexpected antibody whenever subgroup A patient with anti-A1 needs blood transfusion.

Objective

The aim of this study was to reveal the frequency of subgroups of A in Thai blood donors since it has not been established. In addition, the occurrence as well as the reactivity pattern of anti-A1 in A\textsubscript{2}, A\textsubscript{2}B and other A subgroup individuals will also be included in the study.

Materials and Methods

During July 10 to August 5, 2017, among 61,618 blood donations at National Blood Centre, Thai Red Cross Society, Bangkok, Thailand, a total of 17,591 group A blood samples from routine blood processing laboratory were studied. This study was approved by the Human Ethics Committee of National Blood Centre, certificate number NBC 10/2017.

Blood grouping reagents (National Blood Centre, Thai Red Cross Society) used in this study were as follows:

- Anti-A, Lot No. 60022; Anti-B, Lot No. 60013; Anti-AB, Lot No. 59025; Anti-A1, Lot No. 59012; Anti-H, Lot No. 60020; Anti-human globulin (AHG), Lot No. 60011; and A cells, B cells, A\textsubscript{2} cells and Pooled O cells, Lot No. 60070

Exclusion criteria: Samples with transfusion transmitted infectious reactive including HBsAg, HCV, HIV and syphilis were excluded. The study was intended to conduct at a period of one month in order to exclude the repeat donation.

Methods

A\textsubscript{2} subgroup was identified by agglutination technique using monoclonal anti-A1. All A\textsubscript{1} cells but not A\textsubscript{2} cells will agglutinate with anti-A1.

All negative samples which were A\textsubscript{2} and weakly reactive samples with anti-A1 were further retested for ABO typing by cell and serum grouping. These cells were also tested with anti-H. Since A\textsubscript{2} cells express more H antigen as compare to A\textsubscript{1} cells, so this test is useful for the differentiation of some A\textsubscript{2} cells from A\textsubscript{1} cells, in case when weak reaction with anti-A1 was observed. In addition, anti-A1 and unexpected antibody screening were performed by testing all serum samples in this set with A\textsubscript{1}, A\textsubscript{2} and O cells at 4°C, room temperature (RT) at (22°C-24°C), 37°C and antihuman globulin phase (AHG) phase.\textsuperscript{11}

A\textsubscript{3} was identified by observing mixed-field agglutination of cells with anti-A and anti-A,B under microscope.

Results

A total of 13,028 group A and 4,563 group AB were identified in this study. Among group A, the frequencies of A\textsubscript{1}, A\textsubscript{2} and A\textsubscript{3} were 13,000 (99.78%), 23 (0.18%) and 5 (0.04%), respectively. For group AB, the frequencies of A\textsubscript{1}B, A\textsubscript{2}B and A\textsubscript{3}B were 4,483 (98.25%), 59 (1.29%)
and 21 (0.46%), respectively (Table 1). Moreover, among 108 A and AB subgroups, 21.30%, 4.63%, 54.63% and 19.44% of total 108 A and AB subgroups were A\textsubscript{2}, A\textsubscript{3}, A\textsubscript{2}B and A\textsubscript{3}B, respectively (Table 2).

Anti-A\textsubscript{1} detected at RT for A\textsubscript{2}, A\textsubscript{3}, A\textsubscript{2}B and A\textsubscript{3}B was 1 in 23 (4.35%), 1 in 5 (20%), 4 in 59 (6.78%) and 1 in 21 (4.76%), respectively. At RT and 4°C, the occurrence of anti-A\textsubscript{1} was 4 in 23 (17.39%), 1 in 5 (20%), 11 in 59 (18.64%) and 1 in 21 (4.76%) for A\textsubscript{2}, A\textsubscript{3}, A\textsubscript{2}B and A\textsubscript{3}B, respectively (Table 2).

There were 2 samples of A\textsubscript{2}B gave positive unexpected antibody screening, moderate (1+ to 3+) with A\textsubscript{1}, A\textsubscript{2}, and O cells at 4°C only.

Four different patterns of ABO discrepancy in 7 blood samples observed in this study were shown in Table 3. The most common pattern was for A\textsubscript{2}B with weak anti-A\textsubscript{1}. Otherwise, there was only one of each that observed for A\textsubscript{2}, A\textsubscript{3} and A\textsubscript{2}B with anti-A\textsubscript{1}. One A\textsubscript{3}B subgroup clearly showed mixed-field agglutination.

Table 1  Frequency of subgroups in group A and AB blood donors of National blood Centre, Thai Red Cross Society (July 10, 2017 to August 5, 2017)

<table>
<thead>
<tr>
<th>ABO blood group</th>
<th>N (%)</th>
<th>Subgroup</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>13,028 (74.06)</td>
<td>A\textsubscript{1}</td>
<td>13,000 (99.78)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A\textsubscript{2}</td>
<td>23 (0.18)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A\textsubscript{3}</td>
<td>5 (0.04)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>13,028 (100.00)</td>
</tr>
<tr>
<td>AB</td>
<td>4,563 (25.94)</td>
<td>A\textsubscript{1}B</td>
<td>4,483 (98.25)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A\textsubscript{2}B</td>
<td>59 (1.29)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A\textsubscript{3}B</td>
<td>21 (0.46)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>4,563 (100.00)</td>
</tr>
<tr>
<td>Total</td>
<td>17,591 (100.00)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2  Distribution of A and AB subgroups and anti-A\textsubscript{1} detection at RT and RT -4°C

<table>
<thead>
<tr>
<th>A, AB subgroups</th>
<th>N (%)</th>
<th>RT N (%)</th>
<th>RT- 4°C N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A\textsubscript{2}</td>
<td>23 (21.30)</td>
<td>1 (4.35)</td>
<td>4 (17.39)</td>
</tr>
<tr>
<td>A\textsubscript{3}</td>
<td>5 (4.63)</td>
<td>1 (20.00)</td>
<td>1 (20.00)</td>
</tr>
<tr>
<td>A\textsubscript{2}B</td>
<td>59 (54.63)</td>
<td>4 (6.78)</td>
<td>11 (18.64)</td>
</tr>
<tr>
<td>A\textsubscript{3}B</td>
<td>21 (19.44)</td>
<td>1 (4.76)</td>
<td>1 (4.76)</td>
</tr>
<tr>
<td>Total</td>
<td>108 (100.00)</td>
<td>7</td>
<td>17</td>
</tr>
</tbody>
</table>

Table 3  Patterns of ABO discrepancies observed in the confirmatory test of 7 A subgroups with anti-A\textsubscript{1}

<table>
<thead>
<tr>
<th>A, AB subgroup</th>
<th>N</th>
<th>Anti-A</th>
<th>Anti-B</th>
<th>Anti-AB</th>
<th>Anti-A\textsubscript{1}</th>
<th>Anti-H</th>
<th>A\textsubscript{1} cells</th>
<th>A\textsubscript{2} cells</th>
<th>B cells</th>
<th>O cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>A\textsubscript{2}</td>
<td>1</td>
<td>4+</td>
<td>0</td>
<td>4+</td>
<td>0</td>
<td>4+</td>
<td>1+</td>
<td>0</td>
<td>4+</td>
<td>0</td>
</tr>
<tr>
<td>A\textsubscript{3}</td>
<td>1</td>
<td>1+ &quot;m&quot;</td>
<td>0</td>
<td>1+ &quot;m&quot;</td>
<td>0</td>
<td>4+</td>
<td>1+</td>
<td>0</td>
<td>4+</td>
<td>0</td>
</tr>
<tr>
<td>A\textsubscript{2}B</td>
<td>4</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
<td>0</td>
<td>2+</td>
<td>1+ &quot;w&quot;</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>A\textsubscript{3}B</td>
<td>1</td>
<td>3+ &quot;m&quot;</td>
<td>4+</td>
<td>3+ &quot;m&quot;</td>
<td>0</td>
<td>2+</td>
<td>1+ &quot;w&quot;</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

mf = mixed-field agglutination
Discussion

Our results showed that \(A_1\) and \(A_1B\) were the most common subgroups among \(A\) and \(AB\) blood groups, respectively. The frequency of \(A\) subgroups in group \(A\) and \(AB\) individuals in Thai blood donors was 0.21% and 1.75%, respectively which was low as compared to other populations.\(^4\)\(^5\) It is worth mention that in this study, the frequency of \(A_2B\) subgroup in group \(AB\) was approximately 7 times (1.29% : 0.18%) higher than that of \(A_2\) subgroup in group \(A\). The similar finding was also observed in South Indian, South Sudan (African) and Japanese populations, but not in Caucasian populations.\(^12\)\(^15\)

Subgroups of \(A\) and \(AB\) individuals may have anti-\(A1\) in their serum. The presence of anti-\(A1\) in \(A_2\) was higher than in \(A_2\) individuals in most populations including this study.\(^1\)\(^2\)\(^3\)\(^4\)\(^5\) All anti-\(A1\) detected in this study group were cold reacting and rather weak antibodies. This finding was also similar to the other reports.\(^4\)\(^5\)\(^12\)\(^13\)\(^14\) Only one of \(A_1\) and \(A_1B\) individuals possessed anti-\(A1\). The two samples with cold reacting unexpected antibodies observed in the non-\(A_1\) group may most likely be anti-I or anti-HI. However, no further attempt for antibody identification had been performed.

Conclusion

The significant observations in this study were the low frequency of \(A\) subgroups in Thai blood donors as compared to other populations and the imbalance in \(A_2\) and \(A_2B\) phenotype frequencies of \(ABO\) group in Thai blood donors was also observed. In addition, anti-\(A1\) in all \(A\) subgroups found in this study showed no clinical significance.

Acknowledgments

The authors would like to thank Dr. Ubonwan Charoonruangrit, the Director of National Blood Centre, The Thai Red Cross Society for her permission to conduct this study. We would also like to thank the staff of Antiserum and Standard Cells Preparation Section for their kind co-operation during the study.

References
