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Research Article

Frequency analysis of subgroup blood typing and Cis-AB type

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A B S T R A C T

The purpose of this study is to prevent side effects of transfusion by analyzing the frequency of subtype blood types in blood type tests. Of the 19,800 cases, 128 (0.59%) cases were classified as subtypes. The distribution of subtype detection in K University Hospital was classified as Cis-AB (39 cases, 30.5%), A_2 (25 cases, 21.6%), A_2B (24 cases, 20.7%), A_1B (13 cases, 11.2%), A_3 (11 cases, 9.5 cases) %), B_3(9 cases, 7.8%), Ag(3 cases, 2.6%), Ax(1 case, 0.95%), Ay(1 case, 0.9%), Bx(1 case, 0.9%), Bel(1 case, 0.9%). Am, Az, and Bm were not detected at all. These subtype blood types are caused by quantitative and qualitative variants of antigenicity on the surface of red blood cells. Finally, the examiner should select blood that matches the patient's blood type and select blood that is not abnormal in the examination and transfuse it to prevent side effects of transfusion in advance.

Keywords: Transfusion, Cis-AB, antigenicity, side effects, blood subtypes.

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INTRODUCTION

The most important aspect of blood typing is the ABO blood group. Before a blood transfusion can be performed, the blood type is determined by cell typing, which tests for the presence or absence of antibodies and antigens on the patient's red blood cells, and serum typing, which tests for the presence or absence of antibodies on A-cells and B-cells in the patient's serum.[1]

In the case of a mismatch, technical errors in the test are also suspected, but it is also necessary to check the blood type of the subtype.

There are several possible causes of blood group discrepancy: low or absent antigens, quantitative or qualitative abnormalities of antigens due to subgroups or variants of type A and B, resulting in a weaker than normal serologic response (A_2, Am, Ax, B_2, Bx, Cis-AB), gammaglobulinopathy, neonatal, chimera, presence of irregular antibodies, etc.

According to the International Society of Blood Transfusion (ISBT) standards, ABO There are over 500 blood types (systems), but they are generally divided into 4 groups: A, B, O, and AB.

However, in addition to the four blood types, subgroups cause quantitative and qualitative variants in antigenicity on the surface of red blood cells, and show weak agglutination reactions in serological reactions, leading to confusion when determining ABO blood types. These subtypes are mainly present in types A and AB, which not only cause confusion in blood type testing, but also cause acute hemolytic transfusion reactions due to inappropriate transfusion.

For this reason, it is important to look at the causes of ABO discrepancies, categorize the causes of blood and serum problems into false positives and false negatives, and trace the causes to determine the correct ABO blood type.[2]

In the agglutinogen of type A, there are many subtypes of antigens such as A_1, A_2, A_3, Am, Ag, Ax, Az, among which the important subtypes in blood banking practice are the subtypes with A_1, A_2, and A_3. In addition, the classification of AB subtypes is dominated by A_1B, A_2B, and Cis-AB.

Looking at the subtypes of A and AB, which are closely related to blood transfusion, there are four blood types: A_2, A_1B, A_2B, and Cis-AB.

The frequency of type A_2 in Korean people is 0.17~0.44%, type A_2B is 0.58%, and type Cis-AB is 0.78%. Cis-AB has the highest frequency among all blood types. These

subgroups often have an irregular antibody, anti-A_2, in their serum, which causes discrepancies between cell-typing and serum-typing tests.[4]

In order to identify these discrepancies, the principle is to suspect a subgroup and follow up thoroughly when incompatible agglutination occurs during cross-matching of donor and recipient samples. Of course, other subtypes may exist in addition to the Cis-AB type, but there is no need to check them individually as a daily work.[4]

Therefore, the purpose of this study is to analyze the frequency of subtype blood types that cause discrepancies between cell typing and serum typing in subgroups, and among them,

Cis-AB type was analyzed to prevent acute hemolytic transfusion side effects that occur in patients during blood transfusion in advance.[3]

MATERIAL AND METHODS

Materials

1. Specimen

This study was conducted on 19,800 transfusion patients referred from S University Hospital in Gyeongbuk for one year in 2022. Specimens referred to the blood bank were first subjected to cell typing and serum typing using the ABOtyping test, and frequency analysis was performed on specimens with weak agglutination reactions to analyze subgroup types and cis-AB types.

2. Reagents for analysis

Anti-A, Anti-B reagent by ABO typing, subtype (subgroup) test reagents are anti-A_1Lectin (Dolichosbiflorus), Anti-H Lectin (Ulexeuropaeus), Elution (Heat, Ether), Adsorption of Cold-Reactive Autoantibodies, Adsorption of Warm Reactive AutoantibodiesZZAP, Dissociation (chloroquine) method was used to test.[3]

3. Subjects of subgroup blood test

There were 128 (0.59%) of the samples whose blood type was withheld due to weak agglutination reaction in cell typing and serum typing ABO typing, mainly A_2, A_3, A_1B, A_2B, and Cis-AB types. etc. was carried out.

4. Inspection method

a. Test using Anti-A_1 Lectin (Dolichosbiflorus) and Anti-H Lectin (Ulexeuropaeus)

Anti-A_1 Lectin (Dolichosbiflorus plant extract) reagent was used to check the A_1 and A_2 types first to determine the presence or absence of agglutination in the subtype reaction. If agglutination occurred in the A_1 substance, the blood type was determined to be A_1, and if non-agglutination occurred, the subtype was further tested with the Anti-H Lectin (Ulexeuropaeus plant extract) reagent to determine the subtypes of A_1, A_2, A_3, A_1B, A_2B, and Cis-AB.

b. Test using Elution (Heat, Ether) and Dissociation (Dissociation: Chloroquine)

If the blood type determination was ambiguous with Anti-A_1 Lectin and Anti-H Lectin reagents, the final blood type determination was performed by separating the antigen and antibody attached to the blood type using elution (Elution: Heat, Ether) and dissociation (dissociation:chloroquine) reagents.

5. Statistical analysis

Statistical analysis was conducted on 19,800 transfusion patients referred to the blood bank at S University Hospital in Gyeongbuk for one year in 2022, and the data were analyzed using IBM SPSS and Excel programs.

6. Determination Criteria

Based on the aggregation reaction according to ISBT (International Society of Blood Transfusion,) standards, the final determination was made by simultaneously performing a visual determination and a microscopic method.

Table 1: Criteria for determining an agglutination reaction

Aggregation reaction intensity	Aggregation reaction analysis
++++	Visually observed as a single aggregated mass
+++	Visually observed as multiple aggregates
++	Visual observation of large and small aggregates
+	Observation of small aggregates
weak,(+-)	Visually Macroscopic and microscopic microscopic small aggregates (subgroup typing)
m.f(Mixed field agglutination)	A phenomenon in which blood cells are not aggregated and partially aggregated blood cells are present on macroscopic and microscopic images (so-called mixed visual reaction)
O(-)	Visual and microscopic appearance of no aggregation at all

RESULTS

In ABO typing, of the 19,800 specimens, 128 specimens with poor agglutination and undetermined results were subgroups, representing a subtype rate of 0.59%. Subtyping was performed using Anti-A_1 lectin, Anti-H lectin, Elution-Heat-Ether and Dissociation-chloroquine.

Since the blood type test is based on the difference in receptor, which is the antigenic determinant of agglutination strength, the blood type was first determined by checking the strength of antigenic agglutination using Anti-A_1 lectin reagent, and the blood type that showed weak agglutination was again agglutinated with Anti-H lectin reagent (Table 2).

In particular, poor aggregation in the anti-H lectin reagent is ambiguous to determine, so antigenicity is destroyed using the elution (Elution: Heat, Ether) method, and the presence or absence of antibodies is confirmed using serum typing.[7] Also, the presence or absence of antigen was confirmed by the dissociation (Dissociation: chloroquine) method, and a cell typing test was performed to make the final decision.[8]

Table 2: Comparison of Cohesive Strength Responses of ABO Subtypes

Subtype Blood Group Cohesion Strength (subgroup agglutination)								
Phenotypes	cell typing					serum typing		
	anti-A	anti-B	anti-A1	anti-A,B	anti-H	A cell	B cell	O cell
A_2	4+	0	0	4+	2+	0	4+	0
A_3	1+mf	0	0	1+mf	3+	0	4+	0
Am	0	0	0	0	0	0	0	0
Ax	W+	0	0	+	4+	W+	4+	0
Ael	0	0	0	1+mf	4+	1+	4+	0
B_3	0	1+mf	0	0	3+	4+	0	0
Bm	0	0	0	0	0	0	0	0
Bx	0	W+	0	+	4+	4+	W+	0
Bel	0	0	0	4+	4+	4+	1+	0
AxB	W+	4+	0	4+	2+	W+	0	0
A_1BX	4+	W+	4+	4+	2+	0+	W+	0
AmB	0	4+	0	4+	2+	0	0	0
A_1Bm	4+	0	4+	4+	2+	0	0	0
$A_{el}B$	0	4+	0	4+	3+	1+	0	0
A_1B_{el}	4+	0	4+	4+	3+	0	1+	0
A_1B	4+	4+	4+	4+	3+	0	1+	0
A_2B	4+	4+	4+	4+	3+	0	2+	0
A_3B	2+	4+	0	4+	4+	0	4+	0
Cis A_2B	4+	4+	0	4+	3+	3+	0	0
Cis A_2B_3	4+	1+	0	4+	3+	W+	2+	0
Cis A_1B_3	4+	W+	4+	4+	2+	0	4+	0

In the test of subtype blood types (Table 2), the detection distribution shows that Am, Az, and Bm blood types were not detected, and other subtype groups were relatively diverse. When anti-A and A blood cells were reacted, it was found that the agglutination strength was relatively stronger in the subtype with A, and the agglutination strength of the subtype with AB was weaker than that of the A subtype. In the cis-AB subtype with AB, errors in blood type determination may occur and careful judgment is required.[8] The most common subtype detected in this study (Table 3) was Cis-AB (39 cases, 30.5%), followed by A_2 (25 cases, 21.6%), A_2B (24 cases, 20.7%), A_1B (13 cases, 11.2%), A_3 (11 cases, 9.5%), and B_3 (9 cases, 7.8%). There were a few other detections of Ag (3 cases, 2.6%), Ax (1 case, 0.95%), Ay (1 case, 0.9%), Bx (1 case, 0.9%), and Bel (1 case, 0.9%). On the other hand, Am, Az, and Bm (0 cases, 0%) were never detected.

Table 3: ABO subgroup systems

No. system name	Frequency	Percentage(%)
A_2	25	19.5
A_3	11	8.6
Ax	1	0.8
Am	0	0.0
Ag	3	2.3
Ay	1	0.8
Az	0	0.0
A_1B	13	10.2
A_2B	24	18.8
B_3	9	7.0
Bx	1	0.8
Bm	0	0.0
Bel	1	0.8
Cis-AB	39	30.5
Total	128	100.0

In particular, a lot of Cis-AB type was detected, which is a variant type that does not follow the genetic law. Cis-AB is inherited because the genetic factor on one chromosome of both parents is biased to one side. Parents with AB and O cannot have children with AB or O genes.

Cis-AB type is a typical subgroup blood type that causes a lot of confusion in clinical practice because the genetic factors of one of the chromosomes of both parents are biased as they are, so the offspring can have type AB or type O.

Cis-AB is a typical subgroup of blood types that causes a lot of confusion in clinical practice because the genetic factors on one chromosome of both parents are skewed, resulting in either AB or O children. In particular, Cis-AB is an ABO discrepancy blood type and often has an irregular anti-B antibody, so it shows incompatibility in cell typing and serum typing for compatibility. In particular, they often show a mixed field pattern, so visual and microscopic observation must be performed to determine.

DISCUSSION

As mentioned above, the frequency of A_2, which is mainly found in Korean people, is 0.17-0.44%, A_2B is 0.58%, and Cis-AB is 0.78%, and the frequency of subgroups among all blood types is about 1.08%. As shown in the test results, 128(0.59%) of the 19,800 cases at S University Hospital were classified as subtype blood types, which is lower than the average detection rate in Korea.

However, although it is not the average detection rate in Korea, 128 out of 19,800 specimens (0.59%) are detected as subtypes, which is not a small number. In other words, about 10 subtypes are detected out of 1,734 cases per month on average.[9]

The subtype detection distribution in S University Hospital was Cis-AB (39 cases, 30.5%), A_2 (25 cases, 21.6%), A_2B (24 cases, 20.7%), A_1B (13 cases, 11.2%), A_3 (11 cases, 9.5%), B_3 (9 cases, 7. 8%), Ag (3 cases, 2.6%), Ax (1 case, 0.95%), Ay (1 case, 0.9%), Bx (1 case, 0.9%), Bel (1 case, 0.9%), and Am, Az, and Bm were not detected at all.

In blood transfusion, blood type must be accurately determined as a basic test for safe transfusion to the patient. However, in clinical practice, it is often difficult to determine blood type in neonates, elderly patients, cancer patients, immunodeficiency patients, etc. due to antigen deficiency and the presence of irregular antibodies, which are often incompatible with cell typing and serum typing.

According to the American Association of Blood Banks (AABB), among the common subtypes, 2-7% of A_2 sera and 20-28% of A_2B sera are highly subtyped. At S University Hospital, cis-AB was found to be the most prevalent subtype, with A_2B being the most prevalent subtype. Due to this genetic mismatch, the antigen of type A has a strong agglutination reaction normally, but the antigen of type B often agglutinates late with mf (partial agglutination), so it can be misdiagnosed as type A, so careful judgment is required.

The agglutination strength of the subtypes that are important in blood banking is in the order of $A_1B > A_2 > A_2B > A_3$, and the lower the number of antigens, the more likely it is to be misdiagnosed. In this study, the blood types that were determined to be subtypes showed a decrease in the number of antigens on red blood cells and a tendency to show a weak agglutination reaction in the serum reaction. These samples were tested for the presence or absence of antigenic antibodies by absorption, elution, and dissociation (chloroquine) tests.

CONCLUSIONS

The purpose of this study was to find out how much blood subtypes were detected in blood typing tests and to prevent transfusion side effects in advance by analyzing the reactions on the tests. Of the 19,800 subject samples, 128 cases were classified as subtypes, which means that about 10 subtypes are detected out of a monthly average of 1,734 cases. It cannot be said that this number of cases is low in frequency of detection as a subtype.

In the experiment, the agglutination reaction pattern showed that when anti-A and A blood cells were reacted, the agglutination strength was relatively stronger in the subtype with A, and the subtype with AB showed weaker agglutination strength than the subtype with A. Therefore, it was found that the blood type determination error could occur in the subtype with AB. It was confirmed that the subtype that is cis-AB with AB among the blood types can make an error in blood type determination.[13-14]

Cis-AB is a common subgroup of blood types that can be clinically diagnosed as either AB or O, causing much confusion. In particular, Cis-AB is a blood type that is discordant with ABO blood and often has irregular anti-B antibodies, resulting in discrepancies in cell typing and serum typing in compatibility tests.[10]

Therefore, if the blood typing test shows a mixed field pattern, visual and microscopic observations must be performed to determine. In addition, the examiner must accurately determine the blood type of the patient through absorption, elution, and dissociation (chloroquine) tests.[11-12]

The examiner can prevent transfusion side effects in advance by selecting blood that matches the patient's blood type and transfusing blood that has no abnormalities on the test.

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