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A_2 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 A_1 and A_2. About 0.4% of A_2 subgroups and 25% of A_2B have anti-A_1 antibody which agglutinates A_1, but not A_2 cells. *Materials and Method*: In this study conducted in a major blood bank,group-wise analysis of 750donors over three months,with special emphasis on the percentage of A_2 and A_2B individuals was done.Also, identification of A_2 and A_2B subgroup was done in patients. The chance of occurrence group A_2& A_2B patients,transfused with A_1 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-A_1antibody to the transfused A_1 and A_1B red cells. *Results*: Out of the 632A group donors, A_1antigen were present in 574 (90.8%) and A_2 antigen was present in 58 (9.17%)donors. In AB group, 104 were A_1B and 14 were A_2B.A_2 subgroup was found in 9.17 % of A and 13.46% of A_2B. Out of 86A & 14AB group blood patients, 6A_2 and 2 A_2B patients were found and ICT on these patients revealed that 5 A_2 and 1 A_2B patient tested positive for irregular antibodies. *Conclusion*: Routine detection of A_2 subgroup will contribute significantly to the prevention of hemolytic reaction by a natural/acquired anti-A_1 antibody and will also prevent the mistaken transfusion of A_2 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 vitalin transfusion safety. The ABO blood grouping consists of four structural molecules, i.e., A, B, AB, and O[1]. The A antigen has two major subgroups as A_1 and A_2, depending on which A and AB blood groups have been classified. Individuals with A antigen in blood, 20% have A_2subgroupand 80% have A_1 subgroup[2]. Similarly, from individuals with AB antigen in blood 10.3% belong to A_2B and 89.7% belong to the A_1B subgroup[3]. The A_2 subgroup makes up 1% of those encountered in the laboratory and therefore are mainly of academic interest.

A_1 is a very potent gene that creates from 810,000 to 1,170,000 antigen sites on the adult RBC, whereas inheritance of an A_2 gene results in the production of only 240 000 to 290000 antigen sites on the adult A_2 RBC[2]. A_1 and A_2 are differentiated based on an antibody,that is, anti-A_1in the serum. Approximately 0.4% of A_2 subgroups and 25% of the A_2B subgroup possess anti-A_1 antibody[3]. Generally, this antibody reacts below 37ºC (body temperature) and is simply a medical nuisance causing discrepancies in ABO testing and incompatibilities in cross matches with A_1 or A_1B cells.However, this anti-A_1 antibody, when active at 37 ºC, though rare, destroys A_1 cells, leading to transfusion reactions, which has been documented[4]. Thus, the objective of the study is to determine the percentageof $A_2$and A_2B subgroups in the studiedpopulation (donors and patients) and to look for the presence of anti-A_1 in A_2& A_2B donors. The study also aimed to assessA_2 subgroup patients with irregular antibodiesfor safe transfusion practices.

2. Materials and Method

The present study was executed using blood samples of donors and patientsbelonging to blood group A and AB obtained fromthe Department of Blood Bank ina tertiary care hospital in Trivandrum.The period of the studywas 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 $A_2$and A_2B Subgroup and anti-A_1 in A_2 and A_2B

To determine the percentage of A_2 and A_2B subgroups in donors, 750 samples of blood group A and AB of donors were collected. The subgroups were tested using anti-A_1protein[1].Whenever the agglutination was +4 with anti-A antibody but negative with anti-A_1 antibody, the sample was considered to be A_2 subgroup for blood groups A and AB (Table 1).

Table 1: Testing for A_2 and A_2B subgroups

Reaction of red cells with antisera				Reaction of serum with cells			Interpretation
Anti-A	Anti-B	Anti-AB	Anti-A_1	A	B	O	
+4	-	+4	+4	-	+/ H	-	A_1
+4	-	+ 4	-	-o r+/H*	+/ H	-	A_2 *
+4	-	+ 4	+4	-	+/ H	-	A_1B
+4	-	+4	-	-or +/H*	+/ H	-	A_2 B*

H = Hemolysis; - = No agglutination; *Occasionally A_2 and A_2B serum contains anti-A_1 and thus gives reaction with pooled A cells; + to +4 = agglutination of increasing strength

The presence of anti-A_1in A_2 and A_2B subgroups were determined by the tube method[5]. Forward and backward grouping was done using antibodies and the collective A, B and O cells. 1 volume each of anti-A_1 proteinreagent 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

addedto 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 Acells (A_1 and A_2 cells as positive and negative controls should always be included) was added. The contents of each tube were mixed mildly byshaking 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 A_2 and A_2B recipients, 100 multi-transfused patients with 86A & 14 AB blood group were selected. The chance of occurrence group A_2& A_2B patients, transfused with A_1 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 A_2 subgroups being produced immune anti-A_1 antibody to the transfused A_1 and A_1B cells[6]. Serum of A_2 and A_2B groups of patients were taken in separate test tubes and a drop ofculture 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 occurencesof sub-groups 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 A_2 and A_2B Subgroup and anti-A_1 in A_2 and A_2B

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 donorsof the A group, A_1antigen was present in 574 (90.8%)donors and A_2 antigen was present in 58 (9.2%) donors(Table 2). In the case of the AB group, from the 118 donors, A_1B antigen was present in 104 (88.1%) donors and A_2B 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, A_2antigen was found in 72 (9.2% of A group and 11.9% of AB group) donors. In this study, it was found that A_2 as A_2Bamong AB blood group donors was in higher numbersthan A_2among A blood group donors, being statistically significant.From a total of 72 A_2 donors, 2 donors had anti-A_1 among 14 A_2B donors and 1 donor had anti-A_1 among 58 A_2donors(Figure 1).

Table 2: Distribution of A_1, A_2, A_1B & A_2B among donors

Blood group		Subgroup			
		A_1	A_1B	A_2	A_2B
A	Count	574	0	58	0
	% within blood group	90.8%	0.0%	9.2%	0.0%
AB	Count	0	104	0	14
	% within blood group	0.0%	88.1%	0.0%	11.9%
Chi-Square=750.000; p < 0.05					

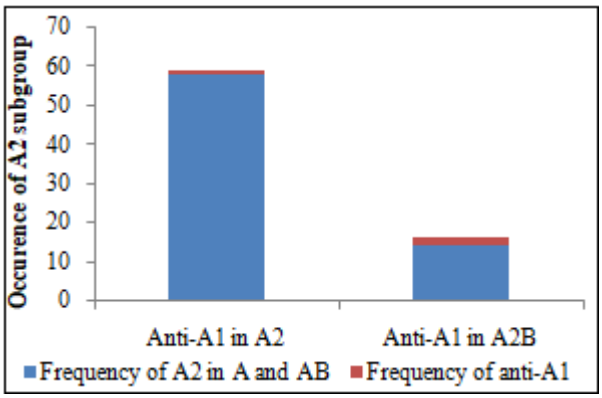

Figure 1: Presence of anti-A_1 among A_2 and A_2B donors

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 blood group patients. From the 86 patients of the A group, A_2antigen was present in 6 (7%) patients and A_1 antigen was present in 80 (93%) patients. In the case of 14 patients of the AB group, A_2B antigen was present in 2(14.3%) patients and A_1B 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, A_2 was present in 8 patients.In this study, it was found that A_2 as A_2B among AB blood group patients was present in higher numbersthan A_2among A blood group patients, found to be statistically significant.

Table 3: Distribution of A_1, A_2,A_1B &A_2B among patients

Blood group		Subgroup			
		A_1	A_1B	A_2	A_2B
A	Count	80	0	6	0
	% within blood group	93.0%	0.0%	7.0%	0.0%
AB	Count	0	12	0	2
	% within blood group	0.0%	85.7%	0.0%	14.3%
Chi-Square=100.000; p<0.05					

The ICT performed on the A_2 and A_2B patients showed that out of 6 A2 patients,5A_2tested positive for irregular antibodies (83.3%)and out of 2A_2Bpatients, 1 A_2B (50%)tested positive for irregular antibodies, being statistically significant (Table 4).

Blood group				Irregular Antibody		Pearson Chi-Square	Sig.
				Nil	Presence of Anti-A_1		
A	Subgroup	A_1	Count	80	0	70.782	p <0.05
			% within Subgroup	100.0%	0.0%		
		A_2	Count	1	5		
			% within Subgroup	16.7%	83.3%		
AB	Subgroup	A_1B	Count	12	0	6.462	p <0.05
			% within Subgroup	100.0%	0.0%		
		A_2B	Count	1	1		
			% within Subgroup	50.0%	50.0%		

The presence of irregular antibody among A2 and A2B have been graphically represented in Figures 2 and 3.

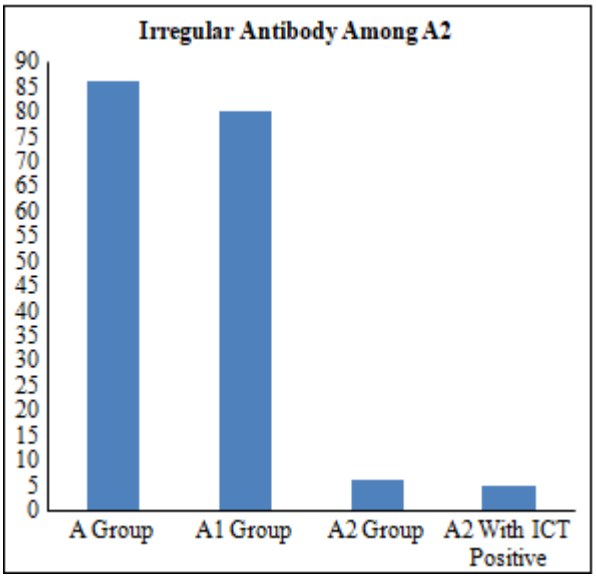

Figure 2: Presence of irregular antibody in A_2subgroup patients

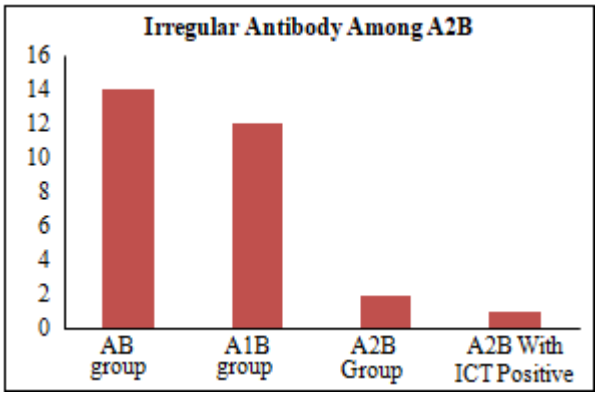

Figure 3: Presence of irregular antibody in A_2B subgroup patient

4. Discussion

The ABO blood profile system with antigens A, B and O has different phenotypic variants (A_1, A_2, B, A_1B, A_2B, and O). The frequency of these variants differs based on different populations[7,8]. In this blood grouping system, group A constitutes 44.6% of all blood groups with 80% prevalence of A_1 and 20% prevalence of $A_2$and AB constitutes the lowest proportion of all blood groups[9].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%[1].In another study fromSouth Gujarat, India, the incidence of A group (24.35%) was lessercompared toO and B groups, while the incidence of AB group was only 8.94%[10].Similarly, in anotherstudy from Sikkim, India, the incidence of A group (22.91%) was lesser compared to O and B groups, while the incidence of AB group wasonly 14.12%[11].

In the present study, the prevalence of A_1 and A_1B was 90.8% and 88.1% and A_2 and A_2B were 9.17% and 11.7% respectively of the donor samples in the blood bank. In a similar study, the prevalence of A_1 and A_1B was 98.3% and 89.7%, while the prevalence of A_2 and A_2B was 0.85% and 1.21%[11]. The present study indicated A_1 as the most common subgroup among A group and A_1B as the most common among the AB group, indicating A_2 and A_2B to be still the uncommon subgroups. A similar distribution was found in the Indian population[12].A similar result was reported in Gwalior, India, where the prevalence of A_2 and A_2B was 8% and 8.6% respectively[8]. In another study, the prevalence of A_2 and A_2B was found to be 4.1% and 19.2%[13].A_1 and A_2constitute the major subtypes of 'A' blood group. A_2 and A_2B are rare subgroup individuals, where a small proportion of A_2 and a higher proportion of A_2B do not recognize A_1 antigens as a part of their own RBC and produce specific anti-A_1 antibody against A_1 cells. Thus, the implication of weaker variants like A_2 is important as they may generate a mistyping with AB as B group and A as O group, thereby triggering hemolysis during transfusion[14].

In the present study, the occurrence of irregular antibody was found to be 14.2% among A_2B donors (2 anti-A_1 out of 14) and was 1.7% among A_2 donors (1 anti-A_1 out of 58). This occurrence of anti-A_1 among the A_2 donors might reflect a past occurrence of blood transfusion, presuming that anti-A_1 antibody in their sera could be natural. In a similar study, 21% anti-A_1 antibody in A_2B donors and 10% anti-A_1 antibody in A_2 donors was reported[8]. However, if the development of antibody to A_1 in A_2 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 A_2patients as A_2B among the AB groupwas significantly higher than the A_2patients among A group, indicating an imbalance in A2 and A2B frequencies in A and AB positive patients. This finding corroborateswith the other studies[1,12,13]. This imbalance might be attributed to the fact that the dominant B

gene might suppress the expression of A_1 antigen, leading to the higher expression of A_2B in the studied population[2]. Further, the present study revealed that anti-A_1 was exhibited by 83.3% A_2 patients (five out of six A_2) and 50% of A_2B patients (one out of two A_2B). Some events can cause unexpected or erroneous serum test result. Patientswith immunodeficiency may not produce a measurable amount of anti-A_1 and anti-B.On the other hand, abnormally high concentrations of anti-A_1 can lead to false-negative results.Anti-A_1, which is reactive *in vitro* at about 30°C but only dubiously active at 37°C, will destroy some of the A_1 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-A_1 has been active at 37°C, extensive destruction of A_1 cells *in vivo* has been recorded[15]. If these people become blood recipients they can have an immediate hemolytic reaction during further transfusion due to a natural/immune anti-A_1 antibody. This points to the fact that by failing to do anti-A_1 agglutination throughout A & AB blood, we fail to detect many A_2& A_2B subgroups and some A_2 subgroup gets detected as O. Also mistaken transfusion of an A_2 subgroup blood (which did not give agglutination with anti-A_1protein and hence misinterpreted as O) to an O recipient can cause immediate hemolysis in the recipient with unpredictable severity, indicating the significance of A_2 subgroup in transfusion and the importance of detecting anti-A_1 in blood grouptesting to differentiate between A_1 and A_2individuals from A and AB groups.

The present duration of the study can be prolonged for years to produce an inclusiveand 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 A_2B subgroup was found to be more prevalent than the A_2 subgroup in the studied population. Both the subgroup A_2 and A_1 reacted with anti-A, however, anti-A_1 antibody differentiated them as it does not react with A_2 cells. Routine detection of A_2 subgroup will contribute significantly to the prevention of hemolytic reaction by a natural/acquired anti-A_1 antibody and will also prevent the mistaken transfusion of A_2 subgroup blood to an O recipient. The study found that anti-$A_1$was not present more in donors, but the testing for anti-A_1should be carried out before blood transfusion to prevent any reaction.

References

[1] Elnour AM, Ali NY, Hummeda SA, Alshazally WY, Elderdery AY, OmerNE. Frequency of the A2-subgroup among blood group A and blood group AB among students of faculty of medicine and health sciences at Alimam Almahadi University, White Nile, Sudan. Hematology and Transfusion International Journal. 2015;1:00022.
[2] Mahapatra S, Mishra D, Sahoo D, Sahoo BB. Study of prevalence of A2, A2B along with major ABO blood groups to minimize the transfusion reactions. International Journal of Scientific Research. 2016;5:189-90.
[3] Giriyan SS, Agrawal A, Bajpai R, Nirala NK. A1 and A2 Sub-Types of Blood Group 'A': A Reflection of their Prevalence in North Karnataka Region. Journal of Clinical and Diagnostic Research. 2017;11:EC40.
[4] Shah K, Delvadia B. 366 The Not So Insignificant Anti-A1 Antibody: Cause of Severe Hemolytic Transfusion Reaction. American Journal of Clinical Pathology. 2018;149:S159-S159.
[5] Mujahid A, Dickert FL. Blood group typing: from classical strategies to the application of synthetic antibodies generated by molecular imprinting. Sensors. 2016;16:51.
[6] Chaudhari CN, Misra RN, Nagpal AK. Transfusion in blood group A2B with anti A1 Recipient. Medical journal Armed Forces India. 2008;64:371.
[7] Mourant AE, Kope AC, Domaniewska K. The distribution of human blood groups and other polymorphisms. 2nd ed. New York, USA: Oxford University Press; 1977.
[8] Sharma DC, Rai S, Iyenger S, Jain B, Sao S. Prevalence and distribution of ABO and Rh-D antigens along with its subgroups & rare types in Greater Gwalior Region. Open Journal of Blood Diseases. 2013;3:69-73.
[9] Reid ME, Lomas-Francis C. The Blood Group Antigen Facts Book. 2nd ed. The Blood group systems and Antigens, Section II. New York: Elsevier Academic Press;2004;23-24.
[10] Raja KA, Dobariya GH, Unagar CA, Pandya AN, Patel JN, Wadhwani SN. Frequency and distribution of ABO and Rh blood groups among blood donors in tertiary care hospital of South Gujarat, India. International Journal of Research and Medical Science. 2016;4:5377-81.
[11] Mathur N, Lamichaney R. A hospital based study to find the distribution of ABO and Rh blood group in the local population of Sikkim, North-Eastern India. The Journal of Medical Research. 2017;3:273-276.
[12] Shastry S, Bhat S. Imbalance in A2 and A2B phenotype frequency of ABO group in South India. Blood Transfusion. 2010;8:267.
[13] Kumar IC, Yashovardhan A, Babu BS, Verma A, Babu KS,Bai DJ. The prevalence of A2 and A2B subgroups in blood donors at a tertiary care teaching hospital blood bank of Rayalaseema region: A pilot study. Journal of Clinical and Scientific Research. 2012;1:50.
[14] Pradhan PK, Mishra SC, Patnaik BK, Mohanty D. Prevalence of ABO and RH Blood Group and Sub-Group A1 and A2 in Blood Donors of Odisha - Experience of One Tertiary Centre. Journal of Blood & Lymph. 2019;9:249.
[15] Rudmann SV. Textbook of Blood Banking and Transfusion Medicine. Philadelphia: Saunders. 1999:72-5. 1999.