

Research Article

A Study of A1 and A2 Subgroup in ABO Blood Group System in Blood Centre of Tertiary Care Hospital

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ABSTRACT

Aim: The aim of the present study was to assess the percentage of subgroup A1 and A2 along with ABO blood group in all donors and recipients in a blood centre of a tertiary care hospital.

Methods: It was a cross-sectional study in which data relating to blood grouping of donors and recipients from January 2023 to December 2023 at Bharati Hospital Sangli Blood Centre were collected and analysed.

Results: Among 9674 cases, highest cases were found of Blood Group O positive 2733 (28.25%) followed by B positive 2672 (27.62%), least prevalent blood group found to be was AB Negative 44 (0.45%). Among 9674 cases, 9044 (93.48%) were Rh positive and 630 (6.52%) were Rh negative blood group. Among 9674 cases, 5806 were blood Group O and B while 3868 were blood group A and AB in which A1 positive were 2566 (26.52%) most prevalent among subgroups while A2B 4 cases (0.04%) was least prevalent blood group among them. Out of 2815 cases of blood Group A, A1 was found in 2746 (97.55%) cases while A2 was found in 69 (2.45%) cases. While out of 1053 cases of Blood group AB, A1B was found in 1003(95.25) cases while A2B was found in 50(4.75%) cases.

Conclusion: The present study showed the most common blood group was O positive while A2 and A2B being the least prevalent blood group. The prevalence of the A1 and A2 subtypes of the blood group is notable among individuals with blood group A and AB. Transfusion of incompatible blood may result in potentially fatal transfusion-related responses.

Keywords: Sub Group A1, A2, ABO Blood Group, Donors, Recipients.

INTRODUCTION

Blood is the liquid connective tissue and the most essential body fluid, which is responsible for the transportation of nutrients, enzymes, and hormones throughout the body. Blood is made up of two components: plasma, which is a fluid and blood cells, which are made up of red blood cells, white blood cells, and platelets. The functional elements of blood that carry gases and nutrition throughout the body are called erythrocytes, or red blood cells. Numerous antigens composed of glycoproteins and glycolipids are present in the complex RBC membrane. A blood group is a classification system for human blood based on the presence of specific markers on red blood cells. These markers are called antigen. ABO is the main and most important blood group system in transfusion and organ transplant medicine. The ABO blood group system was discovered by Karl Landsteiner in 1900¹ based

on presence or absence of A or B antigen on the surface of RBCs. Rh blood group also called as Rhesus blood group is the second most important blood group system in transfusion medicine.

Total 33 blood group system representing over 300 antigens are specified by the International Society of Blood Transfusion.² The genes of these blood group systems are autosomal, except XG and XK which are X-borne and MIC2 which is present on both X and Y chromosomes. ABO antigen is found on human tissue and fluids. The inheritance of ABO gene follows simple Mendelian genetics. ABO is codominant in expression³. It occupies one position or locus on each chromosome 9 is occupied by A, B or O gene. The ABO locus is located on chromosome 9 at 9q34.1-q34.2. It contains 7 exons that span more than 18 kb of genomic DNA. Exon 7 is the largest and contains most of the coding sequence. Exon 6 contains the

deletion that is found in most O alleles and results in a loss of enzymatic activity. The A and B antigen on RBCs is fully developed by 2-4 years of life and then persists for lifetime. The ABO antibodies are mainly IgM type and react at room temperature or colder. ABO antibodies produce strong direct agglutination reaction during ABO testing.

In ABO system, there are four main types of blood groups: A, B, AB, and O. Detection of these four blood groups is based on presence or absence of A and/or B antigens on red cells. According to Landsteiner's law, anti-A and/or anti-B antibodies are always present in plasma of individuals who lack corresponding antigen(s) on their red cells ABO is the only blood group system, in which if an antigen is absent in an individual corresponding antibody is always present in plasma. The phenotypes ABO subgroups are defined on the basis of the differences in the proportion of A and B antigens in red cells and their secretions. The frequency of 'A' subgroup is higher than that of 'B' subgroup. Clinically the most common subgroups encountered are A1 and A2 constituting 80% and 20% respectively. Extremely weak A and B subgroups are rarely encountered but can be diagnosed by the striking discrepancies observed in forward and reverse grouping red cell and serum respectively. Distinction between A1 and A2 subgroups can be done by testing the reactivity of the *Dracontium sibiricum* lectin. *Dolichos biflorus* lectin agglutinates the A1 cells and not A2. A2 phenotype portray the inefficient H → A antigen conversion, A2 red cells show increased reactivity with Anti-H lectin *Ulex europaeus*. There are very few known cases of weak A (and B) subtypes, which studies have shown to exhibit a marked discrepancy between serum and red blood cell grouping results.³ In weaker subgroups of A, anti-A sera produced weaker reactions with subjects having A2 red blood cells than with the people possessing A1 type. Here we report for the first time the identification of the A subgroup A2, A2B and the rest of the major ABO groups in the normal populations.

There is no need for treatment for hemolytic disease of the newborn (HDN) caused by an ABO incompatibility. Fetal hydrops is uncommon, and cases of severe hemolysis requiring exchange transfusions are less frequent. ABO antibody-induced HDN nearly exclusively affects infants born to group O mothers who have blood groups A or B. This is because, although the anti-A and anti-B found

in the serum of group B and A individuals, respectively, tend to be of the IgM type, the anti-A and anti-B generated in group O individuals tend to be of the IgG type (and can therefore cross the placenta). HDN is rare, but it has been documented in babies delivered to mothers with blood groups A2 and B.⁴ In 1941, the Rh blood group system was identified. According to Landsteiner and Wiener⁵, the Rh antibody was nonreactive with 15% of the Caucasian population and agglutinated 85% of human red blood cells. Since it is the principal cause of hemolytic disease of the newborn (HDN), it is of utmost importance in obstetrics. The D antigen is the most immunogenic of the Rhesus antigens. If a person who lacks the D antigen is exposed to it through pregnancy, transfusion, or transplantation, they will develop anti-D.

The aim of the present study was to assess the percentage of sub group A1 and A2 along with ABO blood group in all donors and recipients in blood centre of tertiary care hospital.

MATERIALS AND METHODS

It was a cross-sectional study in which data relating to blood grouping of donors and recipients from January 2023 to December 2023 at Bharati Hospital Sangli Blood Centre were collected and analysed.

The blood samples were collected from donors and recipients. The blood samples were screened for the blood groups ABO and Rh. The A and AB blood groups were further screened and subgrouped into A1, A2, A1B and A2B. Blood grouping (forward and reverse) was performed by tube method as per the departmental standard operating procedures (SOPs). The screening for ABO blood group was done with the help of commercially prepared anti-sera (anti-A and anti-B) Rh antigen was detected using anti-Rh(Dtype)sera. Identification of sub groups A1, A2, A1B and A2B were done by using anti-A1 lectin. Technique followed for blood grouping:

1. Slide Method

Three clean glass slides were taken on which a drop of known anti-sera (anti-A, anti-B and anti-D) were put. A drop of blood sample was added to each one of it. Using the edge of separate slides the blood was properly mixed with the anti-sera. The slides were kept undisturbed for 1-2 minutes at about 37 degrees. The presence of agglutination indicated the presence of that respective blood group and the Rh factor.

For subtyping blood groups, A and AB into A1, A2, A1B and A2B, separate clean glass slides were taken on which a drop of anti-A1lectin and a drop of the blood sample to be tested was taken and mixed properly with the edge of a separate clean glass slide. Presence of any agglutination was observed and the result noted.

For all blood group O samples, presence of H antigen was identified by taking a clean glass slide in which a drop of anti-H lectin and a drop of the blood sample was mixed. Presence of agglutination indicated the presence of H antigen in that blood group. All the samples which were grouped by the slide method were confirmed by the tube method.

2. Tube Method

Forward grouping-Subject's RBCs taken along with a known anti- serum such that the positive result depended on the presence of agglutination in the respective tube or slide.

- 1) A 2-5% suspension of washed RBCs was prepared from the samples to be tested using 0.9% saline as diluents.
- 2) Two test tubes were labelled with the sample number as 'i' and 'ii'
- 3) One drop of each anti-sera A and anti-sera B were place in test tubes i and ii respectively.
- 4) One drop of the RBC suspension was added to both the test tubes.
- 5) After a gentle shake, the test tubes were subjected to centrifugation for 15-20 minutes on a high speed of about 3500 rpm. The RBCs settle down in the test tube in the form of a button or pellet indicating the presence of agglutination.

Reverse grouping-Subject's serum taken and RBCs of known blood group antigens were

added so that a positive result in the tube excluded the presence of that antigen.

- 1) Two test tubes labelled with the sample number were taken and marked as 'i' and 'ii'.
- 2) Two drops of the serum sample were put in each of the test tubes.
- 3) One drop of 'A' RBC reagent and 'B' RBC reagent were added to test tube i and ii respectively.
- 4) After a gentle shake, both the test tubes were subjected to centrifugation for 15-20 seconds at a high speed of about 3500 rpm.
- 5) Agglutination was indicated by formation of RBC button at the bottom of the test tube.

Ethical Clearance

The study protocol was reviewed by The Institutional Ethical Committee and permitted by it, IEC No.BV (DU) MC&H/Sangli/IEC/Dissertation2023-23/449

SAMPLE SIZE CALCULATION FORMULA:
 $Z^2 \cdot p \cdot q / d^2$

Where $z_{\alpha/2}$ =Critical value at 5% level of significance

P=prevalence of Blood group A that is 22.88100 = 0.2288%

Q=1-p =0.7712

D=Margin of error 1% =0.01

N=1.96*1.96*0.2288*0.7712/0.01*0.01 =6779

Inclusion Criteria: All donors and recipients during above mentioned time will be included

Exclusion Criteria: Multiple transfused recipients

Risk Factor: Nil

Facilities: All required facilities were already available

RESULTS

Table 1: ABO & Rh Blood Group Distribution in Present Study

ABO Blood Group with Rh Group	Frequency	Percentage (%)
A POSITIVE	2630	27.19
A NEGATIVE	185	1.91
AB POSITIVE	1009	10.43
AB NEGATIVE	44	0.45
B POSITIVE	2672	27.62
B NEGATIVE	206	2.13
O POSITIVE	2733	28.25
O NEGATIVE	195	2.02
Total	9674	100.00

Among 9674 cases, highest number of case were found of Blood Group O positive 2733 (28.25%) followed by B positive 2672

(27.62%), least prevalent blood group found to be was AB Negative 44 (0.45%).

Table 2: Rh Blood Group Distribution in Present Study

Rh Group	Frequency	Percentage (%)
Rh POSITIVE	9044	93.48
Rh NEGATIVE	630	6.52
TOTAL	9674	100.00

Among 9674 cases, 9044 (93.48%) were Rh positive and 630 (6.52%) were Rh negative blood group.

Table 3: Subgroup Distribution among Cases in Present Study

Subgroup and Rh Group Among Case	Frequency	Percentage	Subgroup	Percentage
A1 POSITIVE	2566	26.52	A1	28.38%
A1 NEGATIVE	180	1.86		
A2 POSITIVE	64	0.66	A2	0.72%
A2 NEGATIVE	5	0.05		
A1B POSITIVE	963	9.95	A1B	10.36%
A1B NEGATIVE	40	0.41		
A2B POSITIVE	46	0.47	A2B	0.52%
A2B NEGATIVE	4	0.04		
OTHERS	5806	60.01	-	-
TOTAL	9674	100.00	-	-

Among 9674 cases ,5806 were blood Group O and B while 3868 were blood group A and AB in which A1 positive were 2566 (26.52%) most

prevalent among subgroups while A2B 4 cases (0.04) was least prevalent blood group among them.

Table 4: Distribution of A1, A2, A1B AND A2B in Blood Group A and AB

A Group	Subgroup	Cases	Percentage%
A Group	A1	2746	97.55
	A2	69	2.45
Total A		2815	100.00
AB Group	A1B	1003	95.25
	A2B	50	4.75
Total AB		1053	100.00

Out of 2815 cases of blood Group A, A1 was found in 2746 (97.55%) cases while A2 was found in 69 (2.45%) cases. While out of 1053

cases of Blood group AB, A1B was found in 1003(95.25) cases while A2B was found in 50(4.75%) cases.

DISCUSSION

Table 5: Comparison of ABO & Rh Blood Group Distribution

Authors	A Positive	A Negative	AB Positive	AB Negative	B positive	B Negative	O Positive	O Negative
Mathur et al ⁶	22.13%	0.78%	14.12%	00	27.86%	0.38%	34.35	0.39
Kumbhakar et al ⁷	24.31%	0.49%	5.39%	0.16%	31.31%	0.69%	35.85%	1.80%

Anchinmae et al ⁸	17.9%	0.8%	4.8%	0.3%	33.4%	1.4%	39.6%	1.8%
Kumar et al ⁹	21.9%	0.79%	5.9%	0.27%	29.3%	1.86%	38.03	1.78%
Giri et al ¹⁰	27.31%	1.07%	8.25%	0.46%	30.54%	1.35%	29.24%	1.74%
Barot et al ¹¹	20.36%	1.42%	7.88%	0.54%	33.75%	2.20%	31.44	2.37%
Satish Kumar et al ¹²	28.42%	1.96%	11.04%	0.66%	29.75%	1.93%	24.30%	1.93
Present study	27.19	1.91	10.43	0.45	27.62	2.13	28.25	2.02

Blood group O Positive (28.25%) was found to be most common blood group in the present study. Similar findings were observed in studies conducted by Mathur et al⁶, Kumbhakar et al⁷, Anchinmae et al⁸, Kumar et al.⁹ Our findings were in contrast to Giri et al¹⁰, Barot et al¹¹ were Blood Group B positive was found to be most

common. Similarly, AB Negative (0.45%) was least common blood group in present study which was in concordance to Mathur et al⁶, Kumbhakar et al⁷, Giri et al¹⁰, Barot et al¹¹, Anchinmae et al⁸, Kumar et al⁹, Satish Kumar et al.¹²

Table 6: Comparison of Rh Blood Group Distribution

Authors	Rh Positive	Rh Negative
Mathur et al ⁶	98.46%	1.56%
Kumbhakar et al ⁷	96.86%	3.14%
Anchinmae et al ⁸	95.7%	4.3%
Kumar et al ⁹	95.2%	4.71%
Giri et al ¹⁰	95.36%	4.64%
Barot et al ¹¹	93.35%	6.55%
Satish Kumar et al ¹²	93.51%	6.49%
Present study	93.48%	6.52

Rh positive blood group was found in (93.48%) cases while Rh negative blood group found to be 6.52%. Similar findings were observed in studies conducted by Mathur et al⁶ (98.46%),

Kumbhakar et al⁷ (96.86%), Giri et al¹⁰ (95.36%), Barot et al¹¹ (93.35%), Anchinmae et al⁸ (95.7%), Kumar et al⁹ (95.2%), Satish Kumar et al¹² (93.51%).

Table 7: Subgroup Distribution

Authors	A%		AB%		B%	O%
	A1%	A2%	A1B%	A2B%		
Kumar N et al ⁹	20.1%	1.1%	8.35%	0.85%	37.8%	31.8%
Meitei S. and Kshatriya G ¹³	16.5%	1.0%	4.9%	00	22.3%	55.33%
Mukhopadhyay R. and Kshatriya G ¹⁴	22.5%	0.83%	11.67%	0.83%	37.39%	24.17%
Gauniyal M ¹⁵	40.69%	1.13%	11.86%	0.57%	22.03%	23.72%
Pattanayak I ¹⁶	38.54%	0.98%	10.24%	0.48%	25.85%	23.91%
Giriyan S, Agrawal A ¹⁸	25.91%	0.30%	7.34%	0.85%	-	-
Present study	28.18%	0.67%	10.03%	0.51%	29.75%	30.27%

In present study O blood group (30.27%) was found to be most common which is in accordance with Meitei S. and Kshatriya G¹³. Kumar N et al⁹. Mukhopadhyay R. and Kshatriya G¹⁴ found that B blood group was most prevalent in their respective study. Gauniyal M¹⁵, Pattanayak I¹⁶

found out that Blood Group A1 was most prevalent blood group in their respective studies. In the subgroups the most prevalent subgroup found was A1 (28.18%) in present

study and A2B (0.51%) least prevalent which is in accordance to all the studies mentioned above.

Table 8: Distribution of Subgroup A and AB

Authors	A1	A2	A1B	A2B
Saboor M et al ¹⁷	90%	10%	66.6%	33.33%
Giriyani S, Agrawal A ¹⁸	98.90%	1.10%	89.70%	10.30%
Elnour AM et al ¹⁹	93.42%	6.58%	91.67%	8.33%
Chaitanya Kumar IS ²⁰	95.9%	4.1%	80.8%	19.2%
Gopal S et al ²¹	91.6%	8.4%	83.1%	16.9%
Kumar N et al ²²	94.8%	5.1%	90.76%	9.23%
Yadav A et al ²³	91.0%	9%	84.37%	15.63%
Sharma DC et al ²⁴	92.0%	8.0%	91.4%	8.6%
Present study	97.55%	2.45%	95.25%	4.75%

Present study showed that A1 (97.55%) was more common in A group and A1B (95.25%) more common in AB which was in agreement with the studies done by Saboor M et al¹⁷, Giriyani S, Agrawal A¹⁸, Elnour AM et al¹⁹, Chaitanya Kumar IS²⁰, Gopal S et al²¹, Kumar N et al²², Yadav A et al²³, Sharma DC et al²⁴. A2 (2.45%) was least prevalent in subgroups which was also seen in studies conducted by Saboor M et al¹⁷, Giriyani S, Agrawal A¹⁸, Elnour AM et al¹⁹, Chaitanya Kumar IS²⁰, Gopal S et al²¹, Kumar N et al²², Yadav A et al²³, Sharma DC et al²⁴. A2B is more prevalent than A2 in all above mentioned studies.

CONCLUSION

The present study showed Blood group O as most frequent while A2 and A2B were the least prevalent blood groups. The prevalence of the A1 and A2 subtypes of the blood group is notable among individuals with blood group A and AB. Transfusion of incompatible blood may result in potentially fatal transfusion-related responses. Consequently, it is crucial to apply A1 & A2 grouping in ABO type to prevent severe transfusion responses. It will result in a general enhancement of blood transfusion procedures.

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