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A Study of A1 and A2 Subtypes Among Whole-Blood Donors With Blood Groups A and AB at the Blood Center of a Tertiary Care Institute in Chhattisgarh

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Abstract

Introduction: The ABO blood group shows various subtypes due to the heterogeneity of A and B alleles. The frequency of these subtypes varies in different populations. Studies related to the frequency of subtypes of blood groups A and AB are lacking in this region. So, we planned this study to estimate the prevalence of A_1 and A_2 subtypes among the healthy blood donor population.

Materials and methods: This was a prospective study performed in the blood center of a teaching hospital in the Chhattisgarh state. Healthy whole-blood donors were included in the study after written informed consent. The conventional test tube method was used for performing forward and reverse blood grouping. Testing with anti-A_1 and anti-H lectin was performed in blood groups A and AB. Additional tests such as saliva testing for secretor status and adsorption-elution were performed if needed.

Results: Four thousand one hundred twelve donor samples were studied, out of which 1170 showed A antigen. Among 1170 samples, 74.6% were blood group A, and 25.4% were AB. Among blood group A, 92.3% were A_1 and 3.3% A_2, and the rest were other subtypes, while in AB, it was 85.2% A_1B and 14.8% A_2B. Two cases of anti-A_1 antibodies were also noted, which were clinically insignificant.

Conclusion: We observed a significantly higher proportion of A_2B than A_2 in our study population. We also found a large proportion of A_{int} in the study participants. Testing with anti-A_1 and anti-H lectin is recommended in blood groups A and AB to determine various subtypes and prevent any incompatibility.

Categories: Pathology, Transplantation, Hematology
Keywords: blood donor, aend, aint, subtype, a2, a1, blood group

Introduction

During the 1900s, Karl Landsteiner discovered the ABO blood group system, which has become the most important system for clinical transfusion medicine. Individuals with blood groups A, B, AB, and O have red blood cells (RBCs) that exhibit A, B, AB, or no antigen, as well as a serum that contains naturally occurring anti-B, anti-A, no antibodies, or both anti-B and anti-A. It is these naturally occurring antibodies that hamper the blood group-incompatible transfusion or transplantation. ABO antigens are produced by adding terminal sugar to an oligosaccharide H chain using blood group-specific transferases, which transfer N-acetyl-D-galactosamine or D-galactose sugar to form either A or B antigens, respectively [1].

Various ABO subtypes have been observed due to the heterogeneity of A and B alleles. These subtypes may present as discrepancies during immunohematological testing. Variable serologic reactivity with human polyclonal anti-A, anti-B, and anti-AB reagents is observed in these subtypes. A_1 and A_2 are the major subtypes encountered in blood group A, which differ both qualitatively and quantitatively from each other. A_1 red cells have $8.1\text{-}11.7\times10^5$ antigenic sites as compared to $2.4\text{-}2.9\times10^5$ antigenic sites on A_2 red cells. Both A_1 and A_2 show strong agglutination by anti-A antiserum. However, anti-A_1 lectin of *Dolichos biflorus* agglutinates A_1 red cells but not A_2 red cells. As the A_2 phenotype reflects the inefficient conversion of H to A antigen, they show increased reactivity with the anti-H lectin of *Ulex europaeus*. A_1 is the most common subtype (80%), followed by A_2 [2]. The lesser observed weak subtypes of blood group A include A_3, A_{end}, A_x, A_m, A_y, and A_{el} observed in <1% [1]. The prevalence of A subtypes varies in different populations and different places. In some populations, such as blacks and Japanese, the frequency of the A_2B phenotype is significantly higher than the expected frequency based on the frequency of the A_2 phenotype [3,4]. In the

southern part of India, the prevalences of A_1, A_2, and other weak subtypes were reported to be 98.14%, 1.85%, and 0.01%, respectively [5]. A hospital-based study performed in Northeastern India showed A_1 to be 98.3% with the rest being A_2 and weak subtypes [6].

On reviewing the literature extensively, no similar study was found regarding the frequency of subtypes in this region. So, we planned this study to determine the prevalence of A subtypes in donors with blood groups A and AB. A part of this study project was previously presented as an abstract in the TRANSMEDCON 2022 conference.

Materials And Methods

This prospective study was conducted in a blood center affiliated with the Department of Transfusion Medicine of a tertiary care teaching hospital in Chhattisgarh between July 2021 and December 2022. The study included 4112 donor samples. After receiving approval from the Institute Ethics Committee of the All India Institute of Medical Sciences, Raipur (approval number: AIIMSRPR/IEC/2O21/699), the study was initiated. Departmental standard operating procedures were followed in the selection of blood donors, which was based on the Drugs and Cosmetics Act, India, with the latest amendments [7]. The study included whole-blood donors who consented to participate. We excluded apheresis donors and therapeutic phlebotomies.

The blood group of the donors was tested by conventional tube technique (CTT). For this purpose, donor blood samples were collected in a vial of ethylenediaminetetraacetic acid (EDTA) at the time of blood donation, after getting informed written consent. The blood group was determined by forward and reverse grouping techniques as per departmental standard operating procedure. Monoclonal antisera anti-A, anti-B, anti-AB, and anti-D (Tulip Diagnostics, Verna, India) were used for forward grouping, and in-house fresh pooled A, B, and O cells were used for reverse grouping. A trained technician performed all the procedures under the supervision of a medical officer following the manufacturers' instructions. Blood groups were determined based on the agglutination pattern in forward and reverse grouping. When the forward and reverse grouping showed coherent results, then only the blood group results were considered valid. Any discrepancy in the forward and reverse grouping was resolved prior to validating the blood group results.

To classify the samples of blood groups A and AB according to their subtype (A_1, A_2, and other subtypes), anti-A_1 lectin was used. Macroscopic agglutination with monoclonal anti-A and no agglutination with anti-A_1 lectin were considered as A_2 subtypes. We also tested O blood groups with anti-A and anti-B antisera to identify weak subtypes of A [8]. The plasma of all the subtypes other than A_1 was further tested with A_1 cells to detect anti-A_1 antibodies. If detected, the thermal amplitude of anti-A_1 antibodies was determined at 4°C, room temperature, and 37°C. Whenever needed, additional testing with anti-H lectin, saliva testing for secretor status, and adsorption-elution studies were performed according to departmental standard operating procedures based on procedures described elsewhere [9]. Figure *1* depicts the workflow for testing samples. In Microsoft Excel (Microsoft Corp., Redmond, WA) spreadsheets, donor demographics, and immunohematology testing data were entered and analyzed.

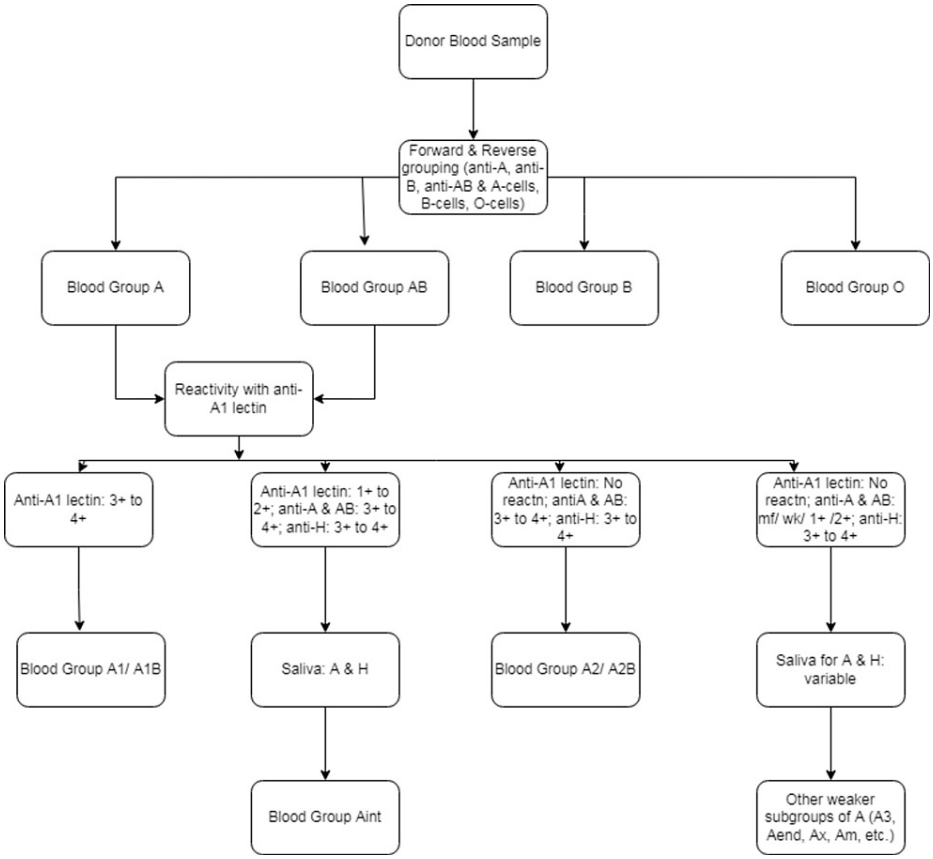

FIGURE 1: Workflow for the testing of subtypes of blood groups A and AB

reactn, reaction; mf, mixed field; wk, weak

Results

The study included 4112 accepted whole-blood donors. The majority (97.5%) of the donors were males (n=4088). Females accounted for only 2.5% as the majority were found ineligible due to low hemoglobin and low weight. Of the donors, 53.5% were 21-30 years, followed by 27.7% in the age group of 31-40 years. We also had 10 participants of >60 years. Of all the donor samples studied, blood groups A, B, AB, and O were found in 21.23% (n=873), 34.46% (n=1417), 7.22% (n=297), and 37.09% (n=1525), respectively. A antigen was present in 1170 donor samples (Table *1*).

A antigen in ABO blood group	Frequency (%)
A	873 (74.6%)
AB	297 (25.4%)
Total	1170 (100%)

TABLE 1: Frequency of blood groups A and AB

On testing with anti-A_1 lectin, A_1 and A_1B subtypes were found in 806 and 253 samples, respectively, while A_2 and A_2B were found in 29 and 44 samples, respectively (Table *2*). We found 37 samples showing intermediate reaction (1-2+) with anti-A_1, for which repeat testing was done with fresh blood samples using antisera of different lots at different temperatures and saliva testing for secretor status. Based on the immunohematological workup, these 37 samples were labeled as blood group A_{int}. We also encountered one blood group A_{end} (Table *3*). Anti-A_1 antibody was found in one case in each of blood groups A_{int} and A_2B, both of which were reactive at room temperature and 4°C but not at 37°C.

ABO blood group	Total number	Subtypes	n (%)	Anti-A_1 antibody
A	873	A_1	806 (92.3)	-
		A_2	29 (3.3)	-
		A_{int}	37 (4.2)	1
		A_{end}	1 (0.2)	-
AB	297	A_1B	253 (85.2)	-
		A_2B	44 (14.8)	1

TABLE 2: Subtypes of blood groups A and AB

Forward grouping						Reverse grouping			Auto-control	Saliva testing	Blood group
Anti-A	Anti-B	Anti-AB	Anti-D	Anti-A_1 lectin	Anti-H lectin	A_1 cells	B cells	O cells			
4+	0	4+	4+	1+	3+/4+	0	4+	0	0	A and H	A_{int}
wk/mf	0	wk/mf	4+	0	4+	0	4+	0	0	H	A_{end} (adsorption-elution showed the presence of A antigen)

TABLE 3: Immunohematological workup for blood groups Aint and Aend

wk, weak; mf, mixed field

We observed that the proportion of A_2B (14.8%) among AB was higher than A_2 (3.3%) among blood group A. On statistical analysis, this difference was found to be of statistical significance ($p<0.05$). The ratio of A_2/A_1 is 0.04, while A_2B/A_1B is 0.17.

Discussion

The frequency of ABO blood groups varies among different populations. A phenotype is found mainly in Northern and Central Europe, while B phenotype is most frequent in Central Asia. Blood group O is the most frequent phenotype globally [10]. Our study included 4112 participants with 1170 having A antigen. Among these, 74.6% were A, and 25.4% were blood group AB, which is similar to the studies performed in North Karnataka [11], as well as Sudan [12].

Blood group A is mainly divided into A_1 and A_2 subtypes based on reaction with anti-A_1 lectin. However, there are several other subtypes such as A_3, A_{int}, A_{end}, and A_m. A_2 and A_2B subtypes are usually less common. Our study findings of A_1 being more common than the A_2 subtype are similar to studies conducted in South Indian and the Sudanese population [5,12]. We found that the prevalence of A_1 and A_2 in blood group A was 92.3% and 3.3%, respectively, whereas that of A_1B and A_2B in blood group AB was 85.2% and 14.8%, respectively. In their study, Giriyan et al. observed the prevalence of A_1 and A_2 to be 98.90% and 1.10%, respectively, and that of A_1B and A_2B to be 89.70% and 10.30%, respectively [11]. Our study findings are similar to that of a pilot study performed by Kumar et al., which showed A_2 and A_2B to be 4.1% and 19.2%, respectively [13].

We observed that the frequency of A_2B in blood group AB as compared to A_2 in blood group A is higher, which was statistically significant. Our findings are similar to studies conducted on blacks and the Japanese population [3,4]. A study conducted by Shastry and Bhat in South India also found A_2B to be significantly higher than A_2 [5]. Usually, the frequencies of A_1 and A_2 phenotypes follow the Hardy-Weinberg equilibrium, but in some populations, such as blacks, Japanese, Chinese, and Indians, the frequency of the A_2B is significantly higher than the expected frequency based on the frequency of A_2 [12]. It could be due to the recessive nature of the A_2 gene compared to the A_1 gene, so a single A_2 gene and B gene show blood group A_2B phenotypically, whereas two A_2 genes or one A_2 gene and one O gene are required for blood

group A_2 [13]. It is also postulated that the higher frequency of the A_2B subtype in these populations could be attributed partially to the reduced synthesis of A_1 substance by the coexisting B enzyme in heterozygous AB individuals [5]. Ogasawara et al. studied ABO alleles by using polymerase chain reaction single-strand conformation polymorphism (SSCP) and nucleotide sequence analyses. It was evident from their study that A_2-related allele frequencies differed between A_2 and A_2B. A putative recombinant allele, R101, was uncommon in individuals with the A_2 phenotype but common in those with the A_2B phenotype. As a result of the study findings, they concluded that R101 is most probably expressed as the A_1 phenotype in R101/O heterozygous individuals but as the A_2 phenotype in R101/B heterozygous individuals, thus giving rise to a high frequency of A_2B phenotypes in R101 heterozygous individuals [4]. The imbalance in the frequencies of A_2B and A_2 subtypes in blacks has been explained by the domination of the *B* gene on the phenol typing expression of A_1B causing this A_1 to be expressed as A_2 or A_{int} leading to A_2B excess [14,15]. We also encountered 37 cases of the A_{int} subtype, which could also be explained by the same reason.

Weaker subtypes of A usually present as group discrepancies. We encountered one case of A_{end}, which is a weak subtype of blood group A. Thakral et al. found that weaker subtypes of ABO resulted in blood group discrepancies in 1:5100 donor samples in their study [16]. Shastry and Bhat, in their study of 40113 samples, found the frequency of weak A subtypes to be 0.01% [5]. All blood group discrepancies should be resolved to rule out any weaker ABO subtype. We encountered anti-A_1 antibodies in one case in each of blood groups A_{int} and A_2B, which were not clinically significant. If clinically significant (reacting at 37°C), they can lead to fatal transfusion reactions. Mishra et al. studied 2874 samples but found only three anti-A_1 antibodies, none of which were clinically significant [17]. It is recommended to perform testing for anti-A_1 antibodies in subtypes other than A_1, especially in settings of ABO-incompatible organ transplantation.

Several case reports regarding A subtypes have been published us [18,19]; however, this is the first study related to the subtypes of blood groups A and AB in this region. The study shows that this region has a significant imbalance of A_2 and A_2B subtypes, which could be due to the presence of *B* gene suppressing the A_1 gene leading to A_{int} and A_2B excess. Molecular studies would have helped, but they were beyond the scope of our study. With rising ABO-incompatible organ transplantations, we recommend mandatory testing of blood groups A and AB with anti-A_1 and anti-H lectin. The importance of subtyping blood group A during incompatible organ transplantation workup has been highlighted by Sachan in a case report from South India [20]. The correct blood typing of donor and recipient samples is needed to prevent any incompatibility. The limitation of the study was that it was performed in an institutional setup, with a limited sample size. We recommend that large population-based studies should be performed in this region to understand the frequency and distribution of various subtypes of the ABO blood group.

Conclusions

This is the first study regarding the prevalence of various subtypes of blood groups A and AB, as well as anti-A_1 antibodies, in this region. We found a significant imbalance in A_2 and A_2B. We recommend immunohematological testing for subtypes and the presence of anti-A_1 antibodies in blood groups A and AB. Population-based molecular studies are suggested to understand the prevalence of subtypes in this region.

Additional Information

Author Contributions

All authors have reviewed the final version to be published and agreed to be accountable for all aspects of the work.

Concept and design: Minal Wasnik, Saurabh Lahare, Ramesh Chandrakar, Nitin Kumar Kashyap

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Drafting of the manuscript: Minal Wasnik, Saurabh Lahare, Ramesh Chandrakar, Nitin Kumar Kashyap

Critical review of the manuscript for important intellectual content: Minal Wasnik, Saurabh Lahare, Ramesh Chandrakar, Nitin Kumar Kashyap

Supervision: Minal Wasnik, Saurabh Lahare, Ramesh Chandrakar, Nitin Kumar Kashyap

Disclosures

Human subjects: Consent was obtained or waived by all participants in this study. The Institute Ethics

Committee of the All India Institute of Medical Sciences, Raipur (IEC-AIIMS Raipur) issued approval AIIMSRPR/IEC/2O21/699. Before commencing the study, ethical approval was obtained from IEC-AIIMS Raipur (approval number: AIIMSRPR/IEC/2O21/699). **Animal subjects:** All authors have confirmed that this study did not involve animal subjects or tissue. **Conflicts of interest:** In compliance with the ICMJE uniform disclosure form, all authors declare the following: **Payment/services info:** All authors have declared that no financial support was received from any organization for the submitted work. **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

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