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Pathology Section

# Less Commonly Encountered Blood Groups: A Cross-sectional Study from A Tertiary Care Centre in North Karnataka Region, India

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#### **ABSTRACT**

**Introduction:** The less commonly encountered blood groups include  $A_1$  negative,  $A_2$  positive,  $A_2$  negative, B negative, O negative,  $A_1B$  negative,  $A_2B$  positive,  $A_2B$  negative, and the Bombay blood group in both Rhesus factor (Rh) positive and Rh negative phenotypes. The frequency of ABO and Rh groups varies across populations and regions.

**Aim:** To examine the prevalence of less common blood groups, namely  $A_1$ -,  $A_2$ +,  $A_2$ -, O-,  $A_1$ B-,  $A_2$ B+,  $A_2$ B-, and Bombay blood group in both Rh positive and Rh negative phenotypes.

**Materials and Methods:** A retrospective, cross-sectional study was conducted over a period of five years from January 2018 to December 2022, involving 36,674 blood donors from a tertiary care hospital at Department of Pathology, SDMCMSH, Dharwad located in the north Karnataka region, India. Blood grouping was performed using the gel card method, and further subtyping was conducted using specific antisera. Results were analysed and presented as frequencies and percentages. All blood groups were tabulated, and the percentages of each blood group were calculated, including  $A_1+$ ,  $A_2+$ ,  $A_1-$ ,  $A_2-$ ,  $A_1B+$ ,  $A_2B+$ ,  $A_1B-$ ,  $A_2B-$ ,  $A_3B-$ ,  $A_4B-$ ,  $A_4$ 

B+, B-, O+, O-, Bombay blood group Rh positive, and Bombay blood group Rh negative.

**Results:** Of the 36,674 donors, 34,803 (94.89%) were Rh positive. The most common blood group was found to be O positive, with 11,897 donors (32.43%), followed by B positive with 10,658 (29.06%),  $A_1$  positive with 9,306 (25.37%), and  $A_1$ B positive with 2,606 (7.10%). The less common blood groups included  $A_1$  negative (467, 1.27%),  $A_2$  positive (45, 0.12%),  $A_2$  negative (8, 0.02%), B negative (614, 1.67%), O negative (614, 1.67%),  $A_1$ B negative (133, 0.36%),  $A_2$ B positive (282, 0.76%),  $A_2$ B negative (35, 0.09%), and the Bombay Oh phenotype (Rh positive) with 9 (0.02%) of the total.

**Conclusion:** This study highlights the prevalence of less commonly encountered and rare blood groups, ensuring adequate maintenance of inventory to meet needs, especially during adverse situations such as road traffic accidents, postpartum haemorrhage, and other medical, surgical, critical care emergencies, and mass casualties involving these rare blood groups.

**Keywords:** A antigen, A<sub>2</sub>, Rh typing, Bombay blood group, Gel card technique

# **INTRODUCTION**

Blood groups in the population are determined genetically due to the presence of specific antigens on the erythrocytes [1]. The ABO blood group system was discovered by Landsteiner in 1901. Individuals have antibodies in their serum that react against red cells lacking the corresponding antigen [2]. There are four main blood groups in this system: A, B, O, and AB [1,3]. The A antigen has two main subtypes, A, and A, based on which blood groups A and AB have been categorized as A<sub>1</sub>, A<sub>2</sub>, A<sub>4</sub>B, and A<sub>2</sub>B. A<sub>4</sub> and A<sub>5</sub> are differentiated based on their reactivity with lectin, with anti A, occurring as a cold agglutinin exclusively against A, cells [3]. Karl Landsteiner and Weiner discovered the Rh blood group system in 1941 [1,4]. The D antigen primarily determines the Rh group as Rh positive or Rh negative [2,4]. Individuals whose red cells lack the D antigen do not typically have anti D in their serum, which contrasts with the ABO blood group system [2]. The frequency of ABO and Rh groups varies across populations and regions [1,2,5]. In view of such variations, studies determining the prevalence of blood groups become important. They not only help in managing blood products but also aid in the assessment of different disorders related to grouping. Dr. Y.M. Bhende discovered the Bombay blood group in 1952. This blood group is characterised by the absence of A, B, and Hantigens on red cells, with the presence of anti A, anti B, and anti H antibodies in serum. It is a rare blood group, and individuals with this blood group must be transfused with the same blood group [6,7].

Less commonly encountered blood groups can be defined as those with a prevalence of less than five percent in the population. These

groups include  $A_1$  negative, B negative, O negative,  $A_1$ B negative,  $A_2$ B negative,  $A_2$ B negative,  $A_2$ B positive, and the Bombay blood group. Given the changing stock positions in blood banks, understanding the prevalence of these less commonly encountered blood groups is crucial for efficient and continuous care, especially during times of need.

The objective of this study was to emphasize the prevalence of less common blood groups, specifically  $A_1$ -,  $A_2$ +,  $A_2$ -, B-, O-,  $A_1$ B-,  $A_2$ B+,  $A_2$ B-, and the Bombay blood group in both Rh positive and Rh negative phenotypes, among all blood donors from a tertiary care hospital in north Karnataka.

#### MATERIALS AND METHODS

This was a retrospective cross-sectional study conducted over a period of five years, from January 2018 to December 2022, involving 36,674 subjects who donated blood at a blood center in a tertiary care hospital at Department of Pathology, SDMCMSH, Dharwad located in north Karnataka, India. All donors visiting the SDM Blood Centre for voluntary blood donation, as well as those donating blood at voluntary blood donation camps conducted by the SDM Blood Centre, were included in the study. Donors who were rejected during screening and physical examination were excluded. IEC Ref: SDMIEC/2023/572.

#### **Study Procedure**

Blood grouping for eligible donors was performed using the column agglutination technique with an ABO/D + Reverse grouping gel

card, utilising monoclonal anti A, anti B, and anti D for Rh factor determination. Forward grouping was conducted within the gel card, while reverse grouping was done with neutral gel for A, and B cells and control cells. Freshly prepared in-house A,B cells and pooled O cells were used for reverse grouping. Gel cards were properly labeled with the patient's name and hospital registration number.

For a 500-microliter sample, 25 microliters of the patient's red cells were added to a test tube to prepare a 5% red cell suspension. Ten microliters of this suspension were added into the first three microtubes. Fifty microliters of A and B pooled cells were added to the reverse grouping microtubes, while 50 microliters of O pooled cells were added to the control microtube. Gel cards were centrifuged for 10 minutes, and results were interpreted.

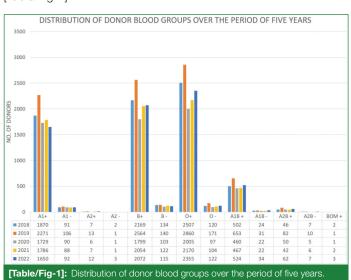
Samples of group A and AB were further tested with anti A1 lectin 1 using the tube method to classify them into A1 and A2 subgroups. If agglutination was positive with anti A antisera but negative with anti A, lectin, the sample was classified as the A, subgroup. Furthermore, in all A<sub>a</sub> and A<sub>a</sub>B blood groups, the presence of anti A<sub>a</sub> antibodies was confirmed at room temperature and 37°C. The Bombay blood group was confirmed using anti-H lectin by the tube method.

#### STATISTICAL ANALYSIS

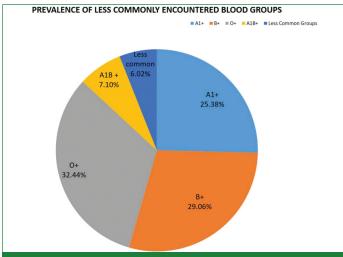
Data were entered into Microsoft Excel and analysed using Statistical Package for the Social Sciences(SPSS) V-21. Quantitative variables were analysed using frequencies and percentages.

# **RESULTS**

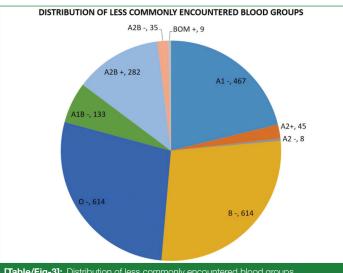
Blood grouping was performed for 36,674 donors over a period of five years using the gel card technique. The prevalence of all blood groups has been summarized in [Table/Fig-1]. Of the total donors, 34,803 (94.90%) were Rh positive and 1,871 (5.10%) were Rh negative. The most prevalent blood group was found to be O positive, with 11,897 donors (32.44%), followed by B positive with 10,658 (29.06%). A, positive and A,B positive groups comprised 9,306 (25.38%) and 2,606 (7.10%) of the total donors, respectively. The less commonly encountered blood groups, such as A<sub>1</sub>-, A<sub>2</sub>+, A<sub>2</sub>-, B-, O-, A<sub>4</sub>B-, A<sub>2</sub>B+, A<sub>3</sub>B-, and the Bombay blood group in both Rh positive and Rh negative phenotypes, comprised 2,207 (6.02%) of the total donors over the studied period, as depicted in [Table/Fig-2].



The specific less commonly encountered blood groups included A, negative (467, 1.27%), A, positive (45, 0.12%), A, negative (8, 0.02%), B negative (614, 1.67%), O negative (614, 1.67%), A,B negative (133, 0.36%), A<sub>2</sub>B positive (282, 0.76%), A<sub>3</sub>B negative (35, 0.09%), and the Bombay Oh phenotype (9, 0.02%) of the total donors. The Bombay Oh phenotype negative blood group was not encountered throughout the entire duration of the study. Among the less commonly encountered groups, the most common were B negative and O negative, while the least common were A2 negative and the Bombay Oh phenotype positive, as shown in [Table/Fig-3].



[Table/Fig-2]: Prevalence of less commonly encountered blood groups of the total



[Table/Fig-3]: Distribution of less commonly encountered blood groups

# DISCUSSION

Marked variations are noted in the distribution of blood groups across different regions, making it necessary to study the occurrence of various blood groups, particularly the less commonly encountered ones, in different areas of the country [1,2]. Various studies have been conducted in different regions to determine the distribution of blood groups, specifically ABO, Rh, Bombay, and other minor blood groups. In this study, a total of 36,674 donors were analysed over five years. Emphasis was placed on the less commonly encountered blood groups, namely the Rh negative phenotypes, groups based on the subtypes of the A<sub>1</sub> antigen (A<sub>2</sub> and A<sub>2</sub>B), and the Bombay Oh phenotype blood group.

Rh positive cases and Rh negative cases in our study were 34,803 (94.90%) and 1,871 (5.10%) respectively. These figures were concordant with the majority of other studies [8-10]. The Rh blood group system comprises 58 different antigenic specificities, with Rh D being the most immunogenic. The presence or absence of the 'D antigen' determines whether an individual is Rh positive or Rh negative, respectively [8,9,11,12].

Blood group O (34.11%) was the most common in this study, and this finding was comparable to other studies, as tabulated in [Table/ Fig-4] [2,7-10,13-17]. However, Chandra T et al., Patel PA et al., and Raja KA et al., reported that blood group B was the most common, with prevalence rates of 39.92%, 39.4%, and 34.43%, respectively. In contrast, our findings indicated that blood group B was the second most common at 30.73% [2,14,15]. A comparison

Blood groups	A (Includes A <sub>1</sub> and A <sub>2</sub> )	В	0	AB	Bombay	Rh positive	Rh negative
Our study, North Karnataka, 2018-22	26.78	30.73	34.11	8.31	0.02	94.9	5.1
Chandra T et al., [2], 2007, Lucknow	21.38	39.92	29.27	9.43	-	95.71	4.29
Mallick S et al., [7], Pondicherry, 2015	-	-	39.9	-	0.008	-	-
Periyavan S et al., [8], South Karnataka (Bangalore), 2000-2007	23.85	29.95	39.81	6.37	0.005	94.2	5.8
Agarwal A et al., [9], Multicentric (Mumbai, Chennai, Nagpur, Dehradun, Kolkata) 2014	20.68	33.07	38.99	6.25	-	94.01	5.4
Das PK et al., [10], Tamil Nadu, 1988-1999	18.85	32.69	38.75	5.27	0	94.53	4.47
Shenoy KDS and Pailoor K [13], South Karnataka (Bangalore) 2018-19	25.47	28.39	38.05	7.62	0.02	94	6
Patel PA et al., [14], Ahmedabad, 2005-11	21.94	39.4	30.79	7.86	-	95.5	4.95
Raja KA et al., [15], Gujarat, 2011-16	24.35	34.43	32.26	8.95	-	95.12	4.87
Geetha R et al., [16], South Karnataka (Bangalore), 2011	-	-	40.5	-	0.01	-	-
Verma A et al., [17], Andhra Pradesh, 2011.	-	-	40.5	-	0.04	-	-

[Table/Fig-4]: A comparison of prevalence of ABO, Rh and Bombay blood groups in present study with other studies [2,7-10,13-17]

Blood Groups	А	В	0	AB	Bombay	Rh positive	Rh negative
Our study, North Karnataka, 2018-22	26.78	30.73	34.11	8.31	0.02	94.9	5.1
Krishna M C et al., [1], Tumkur. Jan-2014 to June 2014	21.54	31.85	37.90	8.71		94.65	5.35
Periyavan S et al., [8], South Karnataka (Bangalore), 2000-2007	23.85	29.95	39.81	6.37	0.005	94.2	5.8
Shenoy KDS and Pailoor K [13], South Karnataka (Bangalore) 2018-19	25.47	28.39	38.05	7.62	0.02	94	6
Geetha R et al., [16], South Karnataka (Bangalore), 2011	-	-	40.5	-	0.01	-	-
Swamy CM et al., [18], Davangere, 2005-2009	26.15	29.85	36.76	7.24		94.48	5.52
Rao C et al., [19], Dakshina Kannada, 2008-2012.	25.8	27.3	42.0	4.8		94.64	5.35
Gadwalkar SR et al., [20], Bellary, 2012-2013	22.04	35.48	34.33	8.49	0.0094	94.94	5.06

[Table/Fig-5]: A comparison of prevalence of ABO, Rh and Bombay blood groups in the present study with other studies of Karnataka [1,8,13,16,18-20].

Blood groups	A <sub>1</sub>	A <sub>2</sub>	A <sub>1</sub> B	A <sub>2</sub> B
Our study, North Karnataka, 2018-22	26.64	0.14	7.46	0.85
Giriyan SS et al., [3], North Karnataka, 2014-2015	25.91	0.3	7.34	0.85
Mahapatra S et al., [21], April to June 2015, Odhissa	20.98	1.3	4.69	2.16
Yazdani MS et al., [22], September to December 2017, Pakistan	21.63	3.26	7.63	1.78

**[Table/Fig-6]:** A comparison of prevalence of  $A_1$ ,  $A_2$ ,  $A_1B$  and  $A_2B$  in present study with other studies [3,21,22].

of prevalence of different blood groups in the present study with other studies of Karnataka has been shown in [Table/Fig-5] [1, 8, 13,16,18-20]. The  $A_1$  and  $A_1B$  blood groups constituted 26.64% and 7.46%, respectively, in this study. A comparison with other studies is made in [Table/Fig-6] [3,21,22].

The reaction of anti  $\rm A_1$  lectin with  $\rm A_1$  cells determines the subtypes  $\rm A_1$  and  $\rm A_2$  of blood group A.  $\rm A_2$  and  $\rm A_2B$  are rare blood groups, and some individuals within these groups have anti  $\rm A_1$  antibodies in their serum against  $\rm A_1$  cells. About 0.4% of  $\rm A_2$  individuals and 25% of  $\rm A_2B$  individuals possess anti  $\rm A_1$  antibodies [3]. The prevalence of  $\rm A_2$  and  $\rm A_2B$  blood groups in this study was 0.14% and 0.85%, respectively. The prevalence of  $\rm A_2$  in group A was lower than the prevalence of  $\rm A_2$  in  $\rm A_2B$ , which is consistent with other studies, as shown in [Table/Fig-3] [3,21]. Although the prevalence of blood group A is more common than that of blood group AB,  $\rm A_2$  is less common than A<sub>2</sub>B.

According to literature from studies conducted across India, the prevalence of B negative and O negative blood types in the population is less than 10 percent. Patidar GK and Dhiman Y reported incidences of 2% and 2.03% for B negative and O negative, respectively [5]. In our study, the prevalence of both these groups was found to be 1.67% each. The prevalence of B negative was consistent with the aforementioned literature, but the prevalence of O negative was lower than reported in these studies.

Understanding the importance of negative blood groups is essential. Rh negative recipients can only be transfused with the corresponding

Rh negative blood type [8,9]. In cases of Rh incompatibility in newborns, including erythroblastosis foetalis, and in patients with Rh weak D positivity, the preferred blood group for transfusion is Rh negative. In emergencies where patients present with massive blood loss and their blood group is unknown, O negative blood can be administered. Due to these factors, understanding the prevalence of Rh negative blood groups, especially O negative, is necessary so that inventory can be maintained according to the anticipated need in that particular region.

The Bombay blood group is the most infrequent blood group, predominantly found in western and southern India, especially within rural populations due to consanguinity [6]. Our hospital in northern Karnataka serves a large rural as well as urban population. Despite this, the prevalence of the Bombay blood group was only 0.02%, highlighting the rarity of its occurrence. A comparison with other studies is depicted in [Table/Fig-4] [7,8,13,16,17].

Individuals with the Bombay blood group have two recessive alleles of the H gene (genotype "hh"), which prevents the expression of the H antigen. Consequently, A and B antigens cannot be produced, explaining the characteristic phenotype of the Bombay blood group. It is the safest type of packed red blood cells (PRBC) that can be transfused to individuals of any blood group. However, a patient with the Bombay blood group should only be transfused with Bombay group blood; otherwise, a lethal transfusion reaction may occur [7,16,17]. A list of Bombay donors should be maintained at every blood center so they can be contacted in cases of emergency need. Additionally, they are advised to refrain from regular, repeated donations to ensure they are available and eligible to donate in times of need.

#### Limitation(s)

This study may have a bias related to the demographic distribution of blood groups as it was conducted at a hospital in north Karnataka, catering to only a section of the entire state's population. Also, this study is based solely on ABO, Rh, and Bombay blood groups and does not include other minor blood group systems that might also play a role in transfusion medicine. It is phenotype-based and

does not consider genetics, which can sometimes show significant variations.

# CONCLUSION(S)

Our study highlights the prevalence of less commonly encountered blood groups, namely  $\rm A_1$  negative,  $\rm A_2$  positive,  $\rm A_2$  negative, B negative, O negative,  $\rm A_1B$  negative, A\_2B positive, A\_2B negative, and Bombay Oh phenotype positive. The Bombay Oh phenotype negative was not encountered during the study period. Among these blood groups, the most prevalent were B negative and O negative. The least common were  $\rm A_2$  negative and Bombay Oh phenotype positive.

Maintaining a sufficient stock at the blood center is one of the most important pillars in a tertiary care center that caters to a large population. This study, by emphasizing the prevalence of less commonly encountered blood groups, helps in deciding and maintaining inventory in anticipation of needs that may arise during emergencies. Therefore, the smooth functioning of the blood center can be ensured even during crises that require less commonly encountered blood groups.

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- iThenticate Software: Apr 12, 2025 (11%)

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- Was informed consent obtained from the subjects involved in the study? No
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