



**Prevalence of blood group antigens other than ABO-rhd in healthy blood donors**

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**Citation this Article:** Dr. Prem Chand Malakar, Dr. Amit Sharma, Dr. Sarita Sharma, Dr. Narendra Dadhich, “Prevalence of blood group antigens other than ABO-rhd in healthy blood donors”, IJMSIR- July - 2022, Vol – 7, Issue - 4, P. No. 243 – 248.

**Type of Publication:** Original Research Article

**Conflicts of Interest:** Nil

**Abstract**

It is important to know the frequencies of the various antigens to predict the availability of blood units for allo immunized patients. Because of the fact that there is always a chance of diversity in phenotype pattern of a donor population, we decided to conduct a study on antigen phenotyping of regular blood donors. Methodology: This blood bank based cross-sectional analytical study was carried out amongst 500 voluntary blood donors. Samples from all these donors were subjected to extended phenotyping (C, c, E, e, K, M, N, S, Jka, Jkb, Fya, Fyb, Lea and Leb). Results: In present study, we observed the percentage frequencies of C, c, E, e, K, M, N, S, P1, Lea, Leb, Jka, Jkb, Fya and Fyb antigens as 75.6%, 53.2%, 18.4%, 97.75%, 3.8%, 82.4%, 58.4%, 43.8%, 66.2%, 16.8%, 52.6%, 80.0%, 67.6%, 79.4% and 54.6% respectively. Conclusion: Outcomes of such studies can be used to formulate a rare blood group donor registry and compatible blood can be provided to the patients (especially those requiring multiple transfusions).

**Keywords:** Antigens, Phenotyping, Blood donors.

**Introduction**

In addition to risks such as transfusion-transmissible diseases (TTD) caused by donor viruses, parasites, or bacterial contaminants of blood products, there is also a risk of alloimmunization due to donor-recipient antigen phenotype disparity. Still, RBCs for blood transfusion are mostly only matched for the major antigens, ABO and D, an approach that is considered safe and cost-effective, except in chronic transfusion recipients (e.g., thalassemia), who additionally require extended matching for minor antigens.<sup>[1]</sup>

The transfusion of ABO compatible but unknown phenotype blood for clinically significant antigens may result in alloimmunization especially in multi-transfused patients.<sup>[2]</sup> It is important to know the frequencies of the various antigens when dealing with patients who have developed multiple alloantibodies. This information is necessary to predict the availability of blood units that lack the corresponding antigen(s). It would be beneficial

to provide antigen negative compatible blood without delay and thus preventing development of transfusion reaction in all immunized patients.<sup>[2]</sup>

At our center total whole blood collection per annum is approximately 35,000 units. Because of the fact that there is always a chance of diversity in phenotype pattern of a donor population, we decided to conduct a study on antigen phenotyping of regular blood donors. The study was based on studying the prevalence and differential expression of the ABO, Rhesus, Kell, Kidd, Duffy, MNS, P, Lewis and Lutheran.

**Material and methods**

This blood bank based cross-sectional analytical study was carried out amongst the voluntary blood donors (both male and female).

Total 500 voluntary blood donors were selected randomly and grouped under 5 cohorts of 100 donors each i.e., Blood group A, B, O, AB and RhD negative. Individuals selected for blood donation as per donor selection criteria according to Drugs & Cosmetics Act, 1940 & Rules, 1945 were included in the study after taking informed consent.

Routine (ABO-RhD) blood grouping of blood donors was done on fully automated immuno-hematology analyzer (Neo Galileo machine) by direct hemagglutination assay using Galileo Grouping Microplates and ABODFULL2 (i.e., 8 well ABO-RhD blood grouping) assay was run using commercially available reagents as per the instrument operator manual. The antigen typing of C, c, E, e, K, M, N, S, Jk<sup>a</sup>, Jk<sup>b</sup>, Fy<sup>a</sup>, Fy<sup>b</sup>, Le<sup>a</sup>, Le<sup>b</sup> was performed by conventional tube method using commercially available antisera, as per manufacturer’s recommendations. The antisera used were gamma-clones (monoclonal or murine monoclonal or monoclonal blend); manufactured by Immucor, Inc,

Norcross, USA. The reaction patterns were recorded. The data was arranged in tabulated and graph forms and analyzed using Epi-info software.

**Observation and results**

Table 1: Sample Selection

Blood Group	RhD+	RhD-	Total
A	100	22	122
B	100	42	142
O	100	23	123
AB	100	13	113
Total	400	100	500

Among the 400 RhD positive samples, 100 samples each were from the blood groups A, B, O and AB. Among the randomly selected 100 RhD negative samples, 22 samples were of A, 42 of B, 23 of O and 13 samples were of AB blood group.

Table 2: Antigenic frequencies of blood groups other than ABO-RhD

Sn	Antigen	Antigens Observed (n=500)	% Frequency
1	C	378	75.6%
2	c	266	53.2%
3	E	92	18.4%
4	e	491	97.75%
5	K	19	3.8%
6	M	412	82.4%
7	N	292	58.4%
8	S	219	43.8%
9	P1	331	66.2%
10	Le <sup>a</sup>	84	16.8%
11	Le <sup>b</sup>	263	52.6%
12	Jk <sup>a</sup>	400	80.0%
13	Jk <sup>b</sup>	338	67.6%
14	Fy <sup>a</sup>	397	79.4%
15	Fy <sup>b</sup>	273	54.6%

Among the total 500 samples of blood donors, 378 were observed to be having C antigen, 266 c, 92 E, 491 e, 19 K, 412 M, 292 N, 219 S, 331 P1, 84 Le<sup>a</sup>, 263 Le<sup>b</sup>, 400 Jk<sup>a</sup>, 338 Jk<sup>b</sup>, 397 Fy<sup>a</sup> and 273 Fy<sup>b</sup> antigen, as summarized in Table 2.

The percentage frequencies of C, c, E, e, K, M, N, S, P1, Le<sup>a</sup>, Le<sup>b</sup>, Jk<sup>a</sup>, Jk<sup>b</sup>, Fy<sup>a</sup> and Fy<sup>b</sup> antigens were calculated to be 75.6%, 53.2%, 18.4%, 97.75%, 3.8%, 82.4%, 58.4%, 43.8%, 66.2%, 16.8%, 52.6%, 80.0%, 67.6%, 79.4% and 54.6% respectively. Antigen e was observed to be having the maximum frequency (491/500; 97.75%) while antigen K was observed to be having the least frequency (19/500; 3.8%).

**Discussion**

In Rh blood group system, extended antigen typing in the study population (n=500) showed the same trend of prevalence of antigens as in other parts of country (i.e., e>C>c>E) as compared in Table 3. In our study,

frequency of e, C, c and E was found 97.75%, 75.6%, 53.2% and 18.4% respectively.

Frequency of Kell antigen (3.8%) was almost similar to the study conducted by Makroo et al [3] and Nanu Thapliyal [4] et al. It has been reported 2-6% in various studies as given in Table 3.

The frequencies of M (82.4%) and N (58.4%) antigens were found comparable to that among other studies (M 75.3-88.8% and N 57-65.4%) while S antigen showed a lower frequency (43.8%) as compared to that among other studies (51-57.8%). [1-6]

Table 3: Comparison of antigenic frequencies among various Indian studies

Antigen	Present Study (n=500) 2019 Bikaner	Makroo et al, <sup>[3]</sup> 2012 (n=3073) Indraprasth Apollo, New Delhi	Thakral et al, <sup>[2]</sup> 2010 (n=1240 for Rh) (n=317 for others) PGIMER, Chandigarh	Nanu & Thapliyal <sup>[4]</sup> 1997	Agarwal N et al, <sup>[1]</sup> 2013 (n=9280 for Rh) (n=508 for others) AIIMS, New Delhi	Lamba DS et al, <sup>[5]</sup> 2013; n=1000; GMC, Chandi-garh	Kahar MA et al, <sup>[6]</sup> 2014 (n=115 Ogrp) (South Gujarat)
C	75.6%	87	84.7	NA	88	85.1	81.7
c	53.2%	58	52.8	NA	56	62.3	56.5
E	18.4%	20	17.9	NA	20	21.5	21.7
e	97.75%	98	98.3	NA	98	99	100
K	3.8%	3.5	5.5	4	2	2.8	6
M	82.4%	88.8	75.3	87	87	88	76
N	58.4%	65.4	61.5	57	63	57.5	62
S	43.8%	54.8	56.7	NA	51	57.8	51
P1	66.2%	72	71.9	NA	NA	NA	64.35
Le <sup>a</sup>	16.8%	NA	18.6	13.3	16.1	NA	16.5
Le <sup>b</sup>	52.6%	NA	60.6	61	24.8	NA	65.2

Jk <sup>a</sup>	80.0%	81.5	82.7	78	76	NA	80.9
Jk <sup>b</sup>	67.6%	67.4	66.5	70	68	NA	71.3
Fy <sup>a</sup>	79.4%	87.4	86.7	83	82	87.3	46.9
Fy <sup>b</sup>	54.6%	57.6	56.1	58	63	58.3	13.9

Antigenic frequencies of P1, Le<sup>a</sup> and Le<sup>b</sup> antigens were also found to be comparable to other studies except that the frequency of Le<sup>b</sup> (52.6%) was observed to be significantly high in comparison to that reported by N. Agarwal et al<sup>[1]</sup> (24.8%). Such variation could be due to antigenic diversity in different populations.

Antigenic frequencies of Jk<sup>a</sup> (80.0%) and Jk<sup>b</sup> (67.6%) were observed to be in line with the frequencies of Jk<sup>a</sup> (76-82.7%) and Jk<sup>b</sup> (66.5-71.3%) antigens among other studies.<sup>[1-6]</sup>

M. Kahar et al<sup>[6]</sup> reported comparatively lower frequencies of Fy<sup>a</sup> and Fy<sup>b</sup> antigens and linked the same with selective pressure due to malarial endemicity (caused by Plasmodium vivax).<sup>[7]</sup>

Variations among frequencies of various antigens, as summarized in Table 3, represent antigenic diversity among different populations. On the other hand, it might be attributed to the selection of study population and methodologies used by the researchers.

Table 4: Comparison of antigenic frequencies (%) among different races worldwide

Antigen	Present Study (n=500) 2019 Bikaner	Caucasian <sup>[8]</sup>	Black <sup>[8]</sup>	White <sup>[9,10]</sup>	Chinese <sup>[11]</sup>
C	75.6%	68	27	78	93
c	53.2%	80	96	80	47
E	18.4%	29	22	29	39
e	97.75%	98	98	98	96
K	3.8%	9	2	8.8	0
M	82.4%	78	74	NA	79.7
N	58.4%	72	75	NA	67.4
S	43.8%	55	31	NA	8.7
P1	66.2%	NA	NA	NA	NA
Le <sup>a</sup>	16.8%	NA	NA	NA	NA
Le <sup>b</sup>	52.6%	NA	NA	NA	NA
Jk <sup>a</sup>	80.0%	77	92	NA	73
Jk <sup>b</sup>	67.6%	74	49	NA	76
Fy <sup>a</sup>	79.4%	66	10	NA	99
Fy <sup>b</sup>	54.6%	83	23	NA	9.2

Well-known differences in the distribution of the blood group antigens among people of different races have been documented, such as those differences between Chinese and Caucasians in Taiwan,<sup>[12]</sup> as well as the differences in the distribution of blood groups in different

ethnic and geographical areas.<sup>[13]</sup> Antigenic frequencies observed in the present study were also compared with the data from different races of Caucasians, Blacks, Whites and Chinese, as given in Table 4.

It was found that C antigen frequency in present study (75.6%) was more than that in Blacks (27%) and lesser than that found in Chinese population (93%).<sup>[8,11]</sup> It was in between Caucasian (68%) and White (78%) populations.<sup>[8-10]</sup> The frequency of c antigen (53.2%) was found closer to antigenic frequency in Chinese population (47%).<sup>[11]</sup> Antigenic frequencies of e (97.75%) antigen was found in accordance with the data from other populations (96-98%)<sup>[8-11]</sup> while Chinese population observed to be showing comparatively higher antigenic frequency of E antigen (39%)<sup>[11]</sup> than that reported among other populations (22-29%),<sup>[8-10]</sup> in our study frequency of E being at lower side, i.e., 18.4%.

The K antigen frequency (3.8%) was found closer to that in Black population (2%).<sup>[8]</sup> The M antigen frequency among our study population (82.4%) was found comparable to that from other populations (74-79.7%) while frequency of N antigen in present study (58.4%) was found lower than others (67.4-75%).<sup>[8-11]</sup> Frequency of S in present study (43.8%) was observed in between the frequencies among Caucasians (55%) and Blacks (31%).<sup>[8]</sup> The antigen frequencies of Jk<sup>a</sup> (80.0%) and Jk<sup>b</sup> (67.6%) were found closest to that of Caucasians (77 and 74% respectively).<sup>[8]</sup> Antigenic frequencies of Fy<sup>a</sup> and Fy<sup>b</sup> were not found comparable to that among any of the races, as shown in Table 4.

### Conclusion

Knowledge of antigenic frequency helps in calculating the number of blood units to be assessed to find an antigen-negative bag or a compatible unit, when required. Outcomes of such studies can be used to formulate a rare blood group donor registry (donor lacking high frequency antigens) at local and national level, and patients (especially those requiring multiple transfusions) with

antibodies against high frequency antigens can be directed to such rare blood group donor registry.

Larger multicentric studies need to be performed to determine antigenic frequencies among different parts of our country to understand antigenic diversity within India, which might also be helpful in making more practical and related panels of screening cells as per Indian requirement for better and more accurate screening and identification of unexpected/ irregular antibodies.

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