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PREVALENCE AND DISTRIBUTION OF ABO AND RH-(D) ANTIGENS IN DELHI –NATIONAL CAPITAL REGION

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Abstract— **The discovery of ABO blood groups by Karl Landsteiner was an important achievement in the history of blood transfusion that was followed by discovery of Rh (D) antigen. The Rh blood group system is one of the most polymorphic and immunogenic systems known in humans. In the past decade, intense investigation has yielded considerable knowledge of the molecular background of this system. Study is aimed to provide data on ABO and Rh-D distribution in Delhi-NCR region and its comparison with the related studies in India and abroad.**

Index Terms— **ABO and Rh (D), blood group antigens.**

Materials and Methods: A total of 3024 anonymous left over whole blood samples were collected from the Indian Red Cross Society, New Delhi to carry out the quality control evaluation of blood grouping reagents at National Institute of Biologicals, Noida, Uttar Pradesh, India .These whole blood samples were grouped serologically for ABO and Rh-D phenotypes using Monoclonal Antisera which were standardized with International Reference Standards from National Institute for Biological Standards and Control (NIBSC),UK.

Results: **Blood group 'B' (32.73%) was the commonest blood group, followed by group O (30.42%), A (24.66%) and AB (12.2%). Rh negativity was observed in 6.58 % and Rh positivity in 93.41 %.**

Conclusion: **Blood group distribution among these blood samples although showed B blood group the most common, blood group O was also found in large number in Delhi NCR region. In phenotypes, R_1R_1 (43%) the most common and R_1r (32%), R_1R_2 (16%) were also found in large numbers. In Rh-D negative, rr (87%) and r'r (8%) phenotype were found among all the blood samples tested.**

Ethical Issue: **For the Quality evaluation of blood grouping reagents, anonymous left over whole blood samples were collected from the Indian Red Cross Society, New Delhi and the protocol for the same has been approved by the Institute Human Ethics Committee. This study was carried out within the acceptable ethical norms.**

Competing interest / Conflict of interest: **The author(s) have no competing interests for financial support, publication of this research, patients and royalties through this collaborative research. All authors were equally involved in discussed research work. There is no financial conflict with the subject matter discussed in the manuscript.**

I. INTRODUCTION

Karl Landsteiner discovered the first and most important blood group system, the ABO blood group system, in 1901. Rh blood group system was the fourth system to be discovered and yet it is second most important blood group from the point of view for transfusion. The ABO and Rh antigens are recognized as the major clinically significant blood group antigens **[1]**. Blood group or blood type is based on the presence or absence of inherited antigenic substance on the surface of red blood cells that can be determined by specific antibodies **[2]**. More than 600 surface antigens have been found on red blood cells **[3]** and several of these antigens that stem from one allele or very closely linked genes collectively form a blood group system **[4]**. The importance of blood group discovery lies in the transfusion of blood amongst different populations irrespective of their ethnic origin, in organ transplantation and in the development of legal medicine, genetic research and anthropology **[5]**.

Human red blood cells contain on their surface a series of glycoproteins and glycolipids, which constitute blood group antigens. Development of these antigens are genetically controlled and they appear early in fetal life and remain unchanged till death **[2]**. The major ABO blood group system is divided into four blood types on the basis of presence or absence of A and B surface antigens and the blood groups are A, B, O and AB. The frequency of four main ABO blood groups varies in the population throughout the world. Blood groups are genetically determined, the vast majorities is inherited in a simple Mendelian fashion and are stable characteristics which are useful in paternity testing **[13].** Blood groups are known to have some association with diseases like duodenal ulcer, diabetes mellitus, urinary tract infection, and Rh incompatibility and ABO incompatibility of newborn **[14].** ABO blood group system derives its importance from the fact that A and B are strongly antigenic and anti A and anti B naturally occurring antibodies present in the serum of persons

lacking the corresponding antigen and these antibodies are capable of producing intravascular hemolysis in case of incompatible transfusion **[6]**.

The Rh blood group system is one of the most polymorphic and immunogenic systems known in humans. Among a total of 29 blood group systems and over 600 different blood group antigens discovered so far, ABO and Rhesus are the most important blood group systems **[7]**. Rh system emerged as second most important blood group system due to hemolytic disease of new-born and its importance in Rh-D negative individuals in subsequent transfusions once they develop Rh antibodies **[8].** Many blood group antigens and their genes have been identified, and their physiological roles uncovered **[1].** Blood group antigens play a vital role in transfusion medicine, genetics understanding, inheritance pattern, and disease susceptibility **[9]**. The benefit of knowledge of the blood group pattern in transfusion services, is useful reducing of maternal mortality rate in clinical practice because in certain conditions an antigen may react with its corresponding antibody and cause serious clinical effects like haemolytic disease of the new-born and haemolytic transfusion reaction **[10]**. Therefore, it is fundamental to have information on the distribution of these blood groups in any population group. In modern medicine besides their importance in evolution, their relation to disease and environment is being increasingly important. It is therefore imperative to have information on the distribution of these blood groups in any population group **[11, 12].**

As the distribution of blood groups like ABO and Rh (D) varies in different populations, the study involved in this field plays a key role in the genetic studies, clinical studies for geographical information thereby reducing morbidity and mortality in blood transfusion services.The present study was done to assess the prevalence of blood groups in Delhi-NCR and to compare our results with other studies conducted in India and abroad, which could be useful in future for the health planners.

II. Objectives

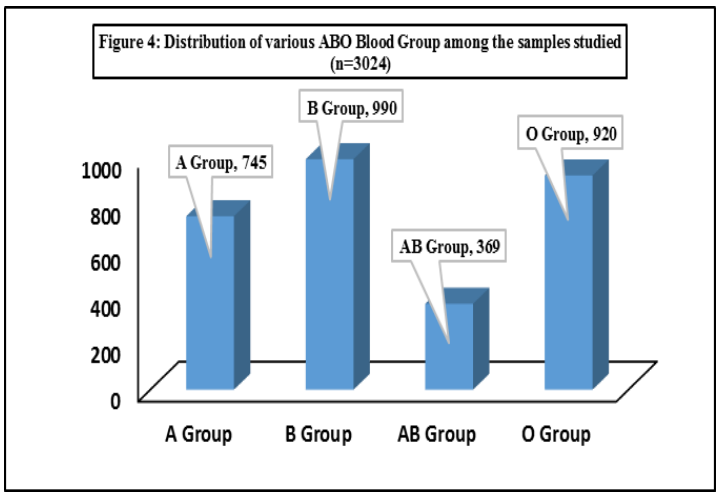
In Blood Transfusion Services, blood, blood components and blood products play a very important role in patients who are given lifesaving critical therapies. This Population study provides data for ABO and Rh (D) Phenotypes among blood donors donating blood at Indian Red Cross Society (IRCS), New Delhi. For the effective management of the blood bank inventory, the prevalence of blood groups like ABO and Rh (D) in different populations is highly essential. Our present study aims at the documentation of the distribution of ABO and Rh blood group in Delhi - NCR region and as well as comparison with the studies conducted within and outside India.

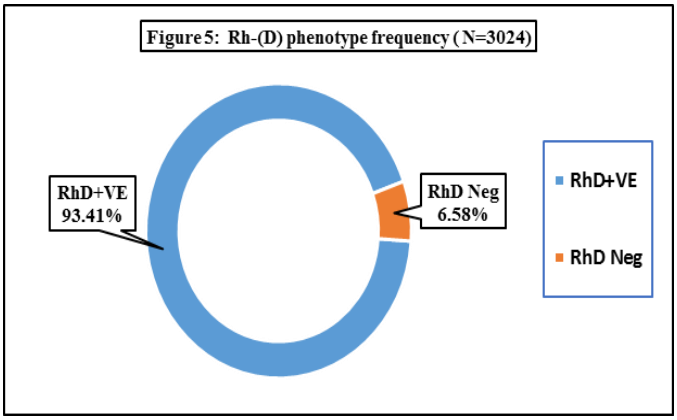
III. Materials and Methods

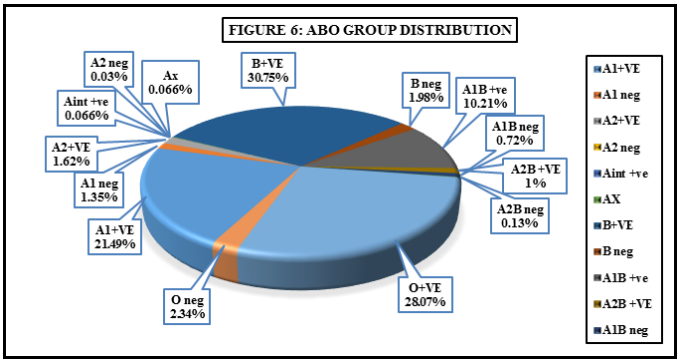
Total of 3024 blood samples were grouped serologically for ABO and Rh-D groups. Anonymous leftover whole blood samples were collected from Indian Red Cross Society, New Delhi. The whole blood samples were grouped and Rh phenotyped serologically by using in-house monoclonal antisera which were standardized with International Reference Standards from National Institute for Biological Standards and Control (NIBSC).Forward grouping and sub grouping of the sample was done by mixing 100µl of 3% red blood cell suspension prepared in normal saline with 100µl of appropriate antisera (Anti -A, Anti B, Anti AB, Anti-A_1 and Anti-H (Lectin). Reverse grouping was done by mixing 100ul of serum of the whole blood sample with 50 µl of known A, B, O red blood cells. Rh typing was done by mixing 50 µl of the 3% red blood cell suspension with 50 µl each of Anti-D (IgG + IgM) from two manufacturers, Anti-C, Anti-E, Anti-c, Anti-e antisera. All the tubes were mixed and centrifuged for one minute at 1000 revolution per minute (rpm) and examined macroscopically and microscopically for confirmation of agglutination. Rh negative blood groups were confirmed for Du positive by using indirect anti-human globulin test.

IV. Results

Out of 3024 blood samples collected, 745 (24.66%) were of Blood group A, 990 (32.73%) of B, 920 (30.42%) of O and 369 (12.20%) were of group AB **(Figure 4)**. Further, the frequency of A positive and A negative was found to be 703(23.34%) and 42 (1.38%) respectively among 3024 blood samples. The frequency of subgroups of A was 691 (22.85%) for A_1, 50 (1.65) for A_2. The weaker variants of group A the frequency reported was 2 (0.066%) and 2 (0.066%) for A_{int} and A_x respectively **(Figure 6)**. Among B group the frequency of B positive was 930 (30.75%) and B Negative was 60 (1.98%). Out of 369 cases of blood group AB reported among 3024 blood samples; 309 (10.21%) were A_1B positive, 22 (0.72%) were A_1B negative, 34 (1.12%) were A_2B positive and 4 (0.13%) were of A_2B negative (Figure 6). The frequency of Rh-D positive was 93.41% (2825) and Rh-D negative was 6.58 % (199) among 3024 blood samples **(Figure 5)**. The prevalence of Rh antigens in D positive (93.41%) was C: 85.78%, c: 59.45%, E: 21.75%, e: 98.18% and further for Rh antigens in D Negative (6.58%) was C: 14.21%, c: 40.54%, E: 78.24%, e: 1.81%.







V. DISCUSSION

ABO blood grouping and Rh typing are essential in blood transfusion, organ transplantation, genetic research, and topological investigations of human ancestral relationships. Literature reviews revealed wide racial as well as geographical variations in ABO and Rh-D phenotypes **[34].** The resultant polymorphism remains important in the population genetic studies, estimating the availabilities of compatible blood, evaluating the possibilities of hemolytic disease of newborn, resolving disputes of paternity/maternity and for forensic purpose **[35, 36].** Distribution of blood groups in India varies regionally with a lot of diversity in various geographical, ethnic and socioeconomic groups. Sound knowledge of frequency of blood group systems is thus highly essential in determining the direction of recruitment of voluntary donors as required for different zones of the country.

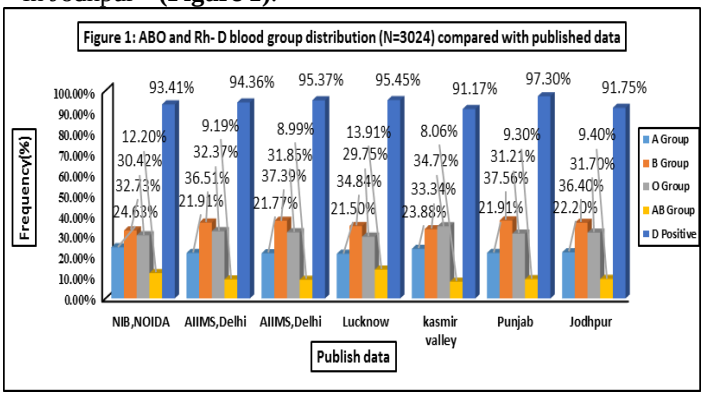
Our study enlightens the prevalence of distribution of ABO and Rh-D blood group systems along with their subgroups in Delhi NCR region and its comparison with the previous studies from India and abroad. The Geographical distribution in India **(Table-1)** depicts 'B' being the commonest blood group in northern and western India whereas in Eastern and Southern India 'O' is the most frequently occurring blood group. So moving from the Southern to Northern zone, the frequency of blood group 'O' is decreasing and blood group 'B' is increasing and western India showed prevalence of blood group B , followed by 'O', Group 'A' and 'AB'. In Northern India, the most prevalent blood group reported is 'B' followed by 'O', 'A' and 'AB'. In the present study the most common group reported in the Delhi NCR is B (32.73%), which is comparable to AIIMS[25] (36.51%), AIIMS[33] (37.39%), Punjab[22] (37.56%), Jodhpur[23] (36.40%), Lucknow[21] (34.84%) and Kashmir[24] (33.34%) in North Indian population. The prevalence of blood group O reported in the present study was (30.42%) which is comparable to Punjab[22] (31.21%), Jodhpur[23] (31.70%), AIIMS[25] (32.37% and 31.85%), Lucknow[21] (29.75%) and Kashmir[24] (34.72%). The frequency of A blood group in the present study was (24.63%) which is close to Kashmir[24] (23.88%) followed by AIIMS (21.91% and 21.77%), Lucknow[21] (21.50%), Punjab[22] (21.91%), Jodhpur[23] (22.20%). The most uncommon blood group in our study reported was AB (12.20%) which is close to Lucknow[21] (13.91%). The frequency of weaker variants of blood group A and AB were A2 (1.65%), A_2B (1.25%), A_x (0.066%), and A_{int} (0.066%).

Table-1 Distribution of ABO and Rh- D antigens in India and Abroad

IN INDIA						
Eastern India						
Place of study	**A**	**B**	**AB**	**0**	**D+**	**D-**
Durgapur (steel city)[15]	23.9	33.6	7.7	34.8	94.7	5.3
Orissa[16]	21.3%	34.16%	7.05%	37.4%	97.63%	2.36%
Western India						
Western Ahmedabad [17]	21.94	39.4	7.86	30.79	95.05	4.95
Eastern Ahmedabad[18]	23.3	35.5	8.8	32.5	94.2	5.8
Surat[19]	24.1	34.89	8.69	32.32	94.18	5.82
Maharashtra[20]	23.38	31.89	8.72	30.99	95.36	4.64
Northern India						
Lucknow[21]	21.73	39.84	9.33	29.1	95.71	4.29
Punjab[22]	21.91	37.56	9.3	31.21	97.3	2.7
Jodhpur[23]	22.2	36.4	9.4	31.7	91.75	8.25
Kashmir valley[24]	23.88	33.34	8.06	34.72	91.17	8.83
AIIMS,DELHI[25]	21.91	36.51	9.19	32.37	94.36	5.64
Delhi NCR (PRESENT STUDY)	24.66	32.73	12.20	30.42	93.41	6.58
South India						
Bangalore[26]	23.85	29.95	6.37	39.82	94.2	5.8
Vellore[27]	21.86	32.69	6.7	38.75	94.5	5.5
Davangere[28]	26.15	29.85	7.24	31.76	94.8	5.2
OUTSIDE INDIA						
Pakistan[29]	22.40%	32.40%	8.40%	30.50%	93%	7%
Nepal[30]	34	29	4	33	96.7	3.33
Britain[31]	42%	8%	3%	47%	83%	17%
USA [32]	41%	9%	4%	46%	85%	15%

In northern India, the comparative publish data in **(Figure 1)** shows that in Kashmir valley[24] (Northern most), blood group O (34.72%) is the most prevalent followed by blood group B (33.34%) and blood group A (23.88%) . The frequency of AB group reported by Lucknow[21] is (13.91%) followed by Delhi-NCR region data (12.20%), AIIMS[25] (9.19%) and AIIMS[33] (8.99%) respectively and least in Kashmir valley (8.06%).

The frequency of D antigen reported in Punjab[22] (97.30%), in Lucknow[21] reported 95.45 %, AIIMS[25] (94.36%), AIIMS[33] (95.37%), our study reported (93.4%), Kashmir[24] population (North most part of India) is 91.17% and 91.75% was reported in Jodhpur[23] **(Figure 1)**.



Rh-D positive frequency was highest in Odisha[16] (97.63%) followed by Punjab[22] (97.3%) and lowest in Jodhpur[23]

(91.75%) **(Table-1)**. In Delhi NCR region, 93.41% Rh-D positive cases were reported but incidence of Rh-D negative in our study was 6.58 % which is more than that observed from other parts of India e.g. Odisha[16] (2.36%), Durgapur[15] (5.3%), Punjab[22] (2.7%), Bangalore[26] (5.8%), Vellore[27] (5.5%), and Davangere[28] (5.2%).

The frequency of Rh-D phenotypes **(Figure-02)** and distribution of different blood groups among 3024 samples (**Table-02)**, the commonest phenotype was R_1R_1 constituting 40.31% of the whole population followed by R_1r (30.15%), R_1R_2 (14.51%), R_2r (5.02%), R_0r (1.42%) and the rarest was R_1R_Z (0.16%) in Rh-D positive samples. In Rh-D negative, the commonest was rr (5.75%) followed by r'r (0.52%), r'r' (0.06%), r''r' (0.19%) and the rarest was r'r'' (0.03%) phenotype were found among all the blood samples tested.

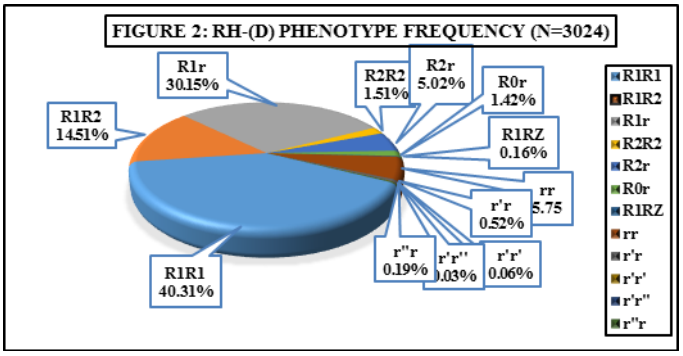
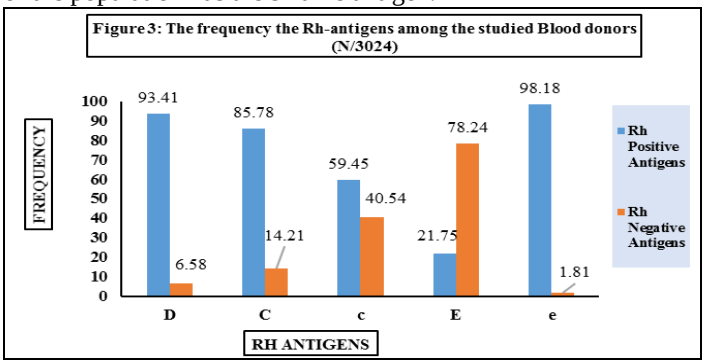


Table-02:

Distribution of Phenotype of different blood groups in Delhi and NCR region															
Blood group	Rh positive	R_1R_1 CCDe.e	R_1R_2 CcDEe	R_1r CcD.ee	R_1R_2 ccD.EE	R_1r ccD.Ee	R_1r ccD.ee	R_2R_2 CCD.Ee	Rh negative	r''r ccdEE	rr ccdee	r'r Ccdee	r'r' Ccdde e	r'r'' CcddEe	Total
A	703	303	112	230	10	39	9	0	42	3	35	4	0	0	746
B	930	401	146	296	21	47	18	1	60	0	55	5	0	0	990
O	849	385	122	264	17	47	11	3	71	3	59	6	2	1	920
AB	343	130	59	122	7	19	5	1	26	0	25	1	0	0	369
Total	2825 (93.4%)	1219	439	912	55	152	43	5	199 (6.58%)	6	174	16	2	1	3024

E is a strong immunogen (almost as strong as D) but this is least effective immunogenic due to its low frequency (antigenicity D>c>E>C>e). The frequency of antigen E (21.75 %) is least common and antigen e is most common (98.18 %) seen in Rh positive people. Further, the frequency of antigen C (85.78 %) and antigen c (59.45 %) respectively. In Rh negative the frequency of antigen C (14.21 %) and c (40.54 %) respectively **(Figure 3)**. Anti-e is often seen as autoantibody and this will make it difficult to find compatible blood since 98.18 % of the population has the small e antigen.



International studies outside India e.g. USA[32], Britain[31] revealed blood group 'O' is the commonest followed by 'A', 'B' and 'AB'. Predominating blood group 'B' in Pakistan[29] while in Nepalese[30] population blood group 'A' as the most prevalent. Whereas studies done in most parts of India reveal commonest blood group being either 'B' or 'O' followed by 'A' and then 'AB'. In Rhesus system our study showed 93.41% were Rh-D positive while only 6.58 % were Rh-D negative which is more similar to the Pakistan blood group frequency. The incidence of Rh-D antigen in most of part of India varies from 91 to 98% for Rh-D positive and 2 to 9% for Rh-D negative. In Britain and USA the distribution of Rh-D positivity is 83% and 85% respectively.

VI. CONCLUSION

We conclude that among ABO blood groups, blood group B is the commonest; followed by O, A in the Northern and western part of India but in Eastern and Southern part O is the commonest ; followed by B and A. The prevalence of A group in present study is higher than the other studies of northern India. We found among the Rh antigens e antigen is the most frequent while antigen E being the least common. In our study, CCD.ee (R1R1) was the commonest phenotype followed by CcD.ee (R_1r) was commonest phenotype type in Rh-D positive samples whereas ccddee (rr) phenotype was commonest in Rh-D negative samples. The generated data in the present regional study will definitely helpful for the health planners for drafting an updated national transfusion policy that will serve to enable them a good insight into the possibilities of future burden of disease with efforts to face further health challenges arising as a consequences of the disease process.

We would like to opine that phenotype and recently genotype showed wide range of variations in different races and religion. Phenotyping and genotyping along with antibody screening and their identification prior to transfusion to patients with the history of multi-transfusion is most vital in transfusion practice in this modern era and this will be helpful in preparing National Rare Donor registry

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