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Study of Prevelance of Rhesus (Rh) Blood Group Antigens Profiling Among Blood Donors

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ABSTRACT

Background : The Rhesus (Rh) blood group literature recognizes 50 antigens in Rh system. Rh blood group antigens D, C E, c, and e are significant in blood transfusion. In practice only ABO and Rh D antigen are done and reported as ABO and Rh positive or Rh negative. Studies have shown blood transfusion reactions have occurred due to Rh E, e and C antigens also. **Aims and Objective**: To determine distribution of 5 Rh antigens- D, C,E, c and e. To generate a database and to provide a compatible blood to recipient without Rh rare antigens that can prevent transfusion reaction in multiple blood transfusions. **Materials & Methods:** 1000 healthy voluntary blood donors selected..Males - 974 and Females - 26. ABO and Rh anti sera used.. **Results:** Frequency of Rh D is 95 %, Rh C is 78.3%, Rh E is 43.7%, Rh c is 21.7 and Rh e antigen is 92.1 % The common Rh antigen is D and least Rh antigen is c. Frequency of Rh phenotypes obtained were DCe (66.2%), DCE(29.1%), Dce (20.6%), DEc (4.2%), dCe (3.8%), dCE (1.5%), dce (1.2%) and dEc (0.2%).Commonest Rh phenotype observed is DCe and the least common is dEc. **Conclusion:** Determination of Rh phenotypes can play a major role in preventing alloimmunization and adverse reactions in multitransfusion and can prevent haemolytic disease of newborns caused by Rh antigens other than Rh D antigen.

Keywords: Rh antigens - Rh D, Rh C, Rh E, Rh c and Rh e , Rh phenotypes, .

I. INTRODUCTION

ABO and Rhesus (Rh) blood group systems are two important blood group systems in transfusion medicine. ABO and Rh blood group antigens are located on Red blood cell surface membrane. Antigens are inherited substances. The Rh antigens are nonglycosylated protein. There are 50 antigens in Rh system. The Rh antigens D, C, E, c and e are the only 5 Rh antigens identified till now and are most significant antigens in blood transfusion [1]. Rh system is controlled by two closely linked loci, RHD gene and RHCE gene. RHD locus carries the gene for RHD polypeptide that expresses Rh D antigen. RHCE locus carries genes for RHCE polypeptide that expresses both C/c and E/e antigens. The RHD and RHCE genes are homologous and are located on chromosome [2,3].

In common practice only ABO and Rh blood grouping are done. In Rh blood grouping only D antigen is done and reported as ABO and Rh positive or Rh negative. There are possibilities of alloimmunization and antibody production in recipients against the Rh blood group antigens other than Rh D antigen even after transfusing ABO and Rh D compatible blood. Studies have shown that blood transfusion reactions had occurred due to presence of antibodies against Rh C, E, c and e antigens. Studies have also shown that hemolytic disease of new borns has occurred due to antibodies against Rh C antigen, E antigen, c antigen and e antigen other than D antigen .[4,5,6,7] Hence determination of different Rh antigens and their phenotypes can play a major important role in preventing alloimmunization and blood transfusion reactions in multi transfusion cases. Rh phenotype in human beings occurs in combination of three antigens (eg. DCe or CcE or dEc or Dce ...). Phenotypes varies in different ethnic groups. The present study is undertaken to determine the frequencies of five Rh antigens viz., D, C, E, c and e and their phenotypes among voluntary blood donors in a particular region.

AIMS OF THE STUDY: 1. To study the prevalence and distribution of different Rh antigens and phenotypes in the blood donors, this can helps to provide a compatible blood for recipient. 2. To determine the percentage of different Rh antigens D, C, E, c, and e. 3. To phenotype all five Rh antigens among blood donors and to generate a

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database of blood donors to prevent future blood transfusion reactions in multiple blood transfusions.

II. MATERIAL AND METHODS

Present study was carried out on 1000 healthy voluntary blood donors, at outdoor blood camps conducted at borders of Karnataka(Kolar Gold Fields) and Andhra Pradesh(Kuppam) states of South India and also at blood banks of Sambhram Institute of Medical Sciences and Research (KGF) and PES Institute Medical Sciences and Research (Kuppam). Study conducted between January 2019–20. .Males were 974(97.4%) and females were 26(2.6%). Male to female ratio is 37.4:1. Age group between 18 and 45 years were considered.

Study design: Prospective-descriptive case study.

Inclusion Criteria: All ABO ,Rh positive and Rh negative blood groups were considered.

Exclusion criteria: Blood donors who were not fit for blood donation were not considered in the study.

After taking donor consent, donors were screened as per WHO protocol. Samples were checked for HIV, HBsAg, HCV, Malarial parasite and VDRL. 5% red cell suspension from blood samples was prepared. Conventional test tube method by using monoclonal antibodies Anti - D, C, E, c and e reagents. Tulip diagnostics Rh anti sera was used **(Fig .1).** For one sample 5 test tubes are taken for Anti-D,Anti-C,Anti-c, and Anti-E, Anti-e Rh Antisera. To 10ul of sample(5% cell suspension), 10 ul of corresponding anti serum is added, mixed well gently, centrifuged at 1000 rpm for one minute and kept in room temp. After 16 seconds, its observed for agglutination macroscopically. If no agglutination macroscopically, a drop of mixture is placed on a slide with a cover slip and observed under microscope. Presence of agglutination indicates Antigen is present. If no agglutination indicates Antigen is absent**.** Grading of agglutinations noted **(Fig.2**).Results are compared with positive and negative controls.

Fig 1. Rh REAGENTS (ANTI-D, ANTI- C, ANTI- E, ANTI- c and ANTI- e

Fig. 2. GRADING OF AGGLUTINATION

Grade 4+ agglutination **Grade 3+ agglitination**

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Grade 2+ agglutination **Grade 1+ agglutination**

Grade 0 – Negative reaction

III. RESULTS

Total of 1000 voluntary blood donor samples of all ABO groups were typed for the presence of Rh (D, C, E, c, e) antigens.

Among ABO blood group the frequency of occurrence of A blood group was 189 (18.9%), B group was 333(33.3%), AB group was 60 (6%) and O blood group was 418(41.8%) ,was most common and AB group is the least. There were 950 (95%) Rh D positive donors and 50 (5%) Rh D negative donors.(**Table 1).**

TABLE 1: NO. OF Rh D POSITIVE AND Rh D NEGATIVE BLOOD GROUPS

Rh D +ve	**950**	**95%**
Rh D –ve	**50**	**5%**

Among Rh positive blood grouping ,occurence of frequencies of 5 Rh antigens are Rh D antigen is 950 (95%), Rh C antigen is 783 (78.3%), Rh E antigen is 437 (43.7%), Rh c antigen is 217 (21.7%) and Rh e antigen is 912 (91.2%). The D antigen was found to be in highest frequency followed by e antigen next common Rh antigen frequency are C and E antigen respectively and the least Rh antigen is c (**Table 2).**

Among 50 Rh negative donors, the highest incidence Rh antigen is "e" (90%). Next common antigen frequencies are C and E (76% and 42 %) respectively and the least common Rh antigen is "c" (24%) **Table 3** . Out of 950 Rh D positive donors the incidence of e antigen is 867 with 86.7%, C antigen is 745 with 74.5 % , E antigen is 416 **. Eight probable phenotypes obtained in the study,they are DCe, DCE, Dce, DEc, dCe, dCE, dce and dEc**. Their frequencies are DCe - 662 (66.2), DCE - 291 (29.1), Dce - 206 (20.6%), DEc - 42 (4.2%), dCe - 38(3.8%), dCE - 15 (1.5%), dce - 12 (1.2%) and dEc – 02 (2%). The most common phenotype among Rh D positive donors is DCe 662 (66.2%) and least common is phenotype is Dec 42 (4.2%). The common phenotype among negative donors is dCe 38 (3.8%) and less common is dEc 02 (0.2%). **Table 4**.

IV. DISCUSSION

The aim of the current study is to know the distribution of 5 different Rh antigens and their phenotypes among blood donors. This would give an idea of prevalance of different Rh antigens in local population. Besides the distribution of Rh antigens and their phenotypes present study was compared with the study conducted from

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Rh antigens	No. of antigens	(%)
D	950	95.0%
C	783	78.3%
E	437	43.7%
c	217	21.7%
e	912	91.2%

TABLE 2: FREQUENCIES OF 5 Rh ANTIGENS AMONG Rh POSITIVE DONORS.

Rh-Phenotypes	Total	(%)
Rh-'e'	45	90 %
Rh-'C'	38	76 %
Rh-'E'	21	42 %
Rh-'c'	12	24 %

TABLE 3. FREQUENCY OF Rh ANTIGENS AMONG Rh NEGATIVE DONORS

N0.	Rh Phenotypes	Frequency	%
1	DCe	662	66.2
2	DCE	291	29.1
3	Dce	206	20.6
4	DEc	42	4.2
5	dCe	38	3.8
6	dCE	15	1.5
7	dce	12	1.2
8	dEc	02	0.2

TABLE 4. INCIDENCE OF Rh PHENOTYPES

Other geographic areas. This study helps to generate and document a data base of donors at Blood Bank Centre, which will be useful in future to issue a compatible blood for blood transfusion to the recipients who require multiple blood transfusions, for example, Thalessemia patients in whom alloimmunization can be prevented. The data on incidence of antigens of various blood groups in the local blood donor population helps in upgrading the blood transfusion practices. The literature gives few studies about the incidence of various Rh blood group antigens of blood donor population from North India.[8,9] The reason for conducting this study was the paucity of studies from the southern state of India.

The prevalence of Rh D positive blood group among population of Chandigarh and North India are 93.4% and 93.0% and Rh negative are 6.6% and 6 % respectively in studies conducted by Thakral et al [8] and Kumar et al [10]. In comparison to the research mentioned above, the prevalence of the Rh D positive antigen (95%) is slightly higher and Rh negative (5%) is slightly less in the present study .

According to a study by Roy et al. [11] 97.8% of blood donors from the East India population were Rh D positive, whereas 2.2% were Rh D negative. When compared to the current study 95% of donors were Rh D positive and 5% were Rh negative. The distribution of Rh D positives in East India is higher, while only 2.2 % of East India participants were Rh D negative. Prevalence of D antigen in present study is slightly higher. In a study conducted by Verma et al [12] shows the occurrence of Rh D antigen is 95.5%. The prevelance of Rh D antigen in present study is same as Verma et al study with only 0.5% difference. Hence incidence of D antigen among

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donor population of Lucknow is same as the present study. Prevalence of Rh D negative blood group in present study is nearly same with the prevalence of Rh D negative in other part of South India in a study conducted by Das et al [13] **(Table5).**

Author	**Year**	**Region**	**No.of donors**	**% RhD +ve donorS**	**% Rh D -ve donors**
Thakral et al[8]	2010	Chandigarh	1000	93.4	6.6
Roy et al [11]	2004	East India	1000	97.8	2.2
Kumar et al [10]	2001	North India	1000	93.0	6.3
Verma et al[12]	2006	Lucknow	1000	95.5	4.7
Das et al [13]	2002	South India	1500	93.3	5.5
Present study	**2019**	**AP & Karnataka border**	**1000**	**95.0**	**5.0**

TABLE 5. COMPARISON OF PREVALENCE OF Rh D ANTIGENS IN BLOOD DONORS IN DIFFERNENT PARTS OF INDIA

A study conducted by Jeremiah ZA et al [14] in Nigeria population observed that the incidence of most frequently occurring Rh antigen was c (99.8%) followed by e (98.7%), D (95%), E (20.5%) and finally C(17.7%) [55]**.** Present study shows same distribution of D antigen.

Jenan Y Taha[15] reported that most frequently occurring Rh antigens among the population in **UAE** was Rh e antigen (97.3%), followed by D (91.1%), C (73.2%), c (71%) and E (21%).The common antigen is' e' and least is 'E' antigen.[56] In the present study most common is D antigen(95%) and the next to it is e antigen (91.2%).

Study conducted by Thakral et al [8] from North India observed that among Rh D positive donors , e was the most common (98.3%), even in the present study, e antigen is common among D postive donors**.** Younis Abed EL [16] studied among 232 students who donated blood in the city of Gaza in Palestine, reported that the percentages of Rh antigen D+, D−, C, c, E and e in the total samples were 92%, 8%, 69%, 81%, 38% and 97%, respectively. In the current study Rh e antigen found to be highest and next highest antigen is D antigen**.** Rh E is least common antigen seen in Rh positive people as only 38% of the population has this antigen[.57] **(Table 6).**

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Rh Ag	Jeremiah ZAetal [14] Nigeria	JenanY Taha [15] UAE	Younis Abel [16] Palestine	Thakral etal[8] North India	Present Study %
D	95.0	91.1	92.0	93.4	95.0
C	17.7	73.2	69.0	84.7	78.3
E	20.5	21.0	38.0	17.9	43.7
c	99.8	71.0	81.0	52.8	21.7
e	98.7	97.3	97.0	98.3	91.2

TABLE 6 COMPARISON OF Rh ANTIGENS FREQUENCIES IN DIFFERENT STUDIES WITH PRESENT STUDY

The prevalence of DCe, DCE, dCe, and dce is considerably lower than that of the current study in a research by Thakral et al. **[42]** and Verma et al **[10].** Occurrence of DCe in the population of India, is most prevalent. Therefore, anti-D, anti-C, and anti-e alloantibodies are thought to be the most widespread Rh blood group system alloantibodies in patients who received blood transfusions. (**Table 7)**

Phenotype	Thakral et al [8] (%)	Verma et al [12] (%)	Present study(%)
DCe	43.8	39.8	66.2
DCE	8.2	11.4	29.1
dCe	07	0	3.8
dce	03	0	1.2

Table. 7 COMPARISON OF Rh PHENOTYPE FREQUENCY AMONG NORTH INDIANS AND PRESENT STUDY

Since D antigen is the most significant antigen of Rh blood group system, there is a clinical importance to the wide variations in D antigen distribution in the different segments of the population. Rh antibodies are thought to be the potential causes of infant hemolytic illness and hemolytic transfusion reactions**[17].**

V. CONCLUSION

According to the study, Rhesus antigenic phenotyping, antibody screening, and their detection before transfusion to patients with a history of multiple transfusions or multiple pregnancies in females are essential in transfusions practise in modern era.

This study will aid in the creation of a donor database for future multipurpose services and a donor database at blood banks for the aim of providing antigen-compatible blood for patients who frequently need blood transfusions, such as, Patients with thalassemia who are most likely to develop alloimuniztion.

The standard practice of ABO and Rh D blood grouping is tested in Blood Banks. Screening for other Rh antigens are not tested which are important in patients who requires multi transfusions. Studies had shown blood transfusion reaction have occured due to Rh antigens other than D antigen and hemolytic disease of new borns have occurred in Rh D positive mother due Anti-C, Anti-E,Anti- c and Anti- e. Hence all Rh antigens detection can be can be considered when there is no any other causes for transfusion reaction.

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