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Original Article

PREVALENCE OF ALLOANTIGEN 'E' IN 'O' POSITIVE BLOOD GROUP DONORS

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ABSTRACT

Background and objective: The antigen 'e' is one of the alloantigen, which can develop alloantibodies in multitransfused patients. Our study objective is to assess the antigen 'e' in the 'O' Blood group donors.

Material and methods: In this study we included 1000 'O' positive blood group donors and their consent was procured. The donors' blood sample was collected after blood donation in Ethylene diamine tetra acetic acid (EDTA). ABO and Rh (D) blood grouping was done using monoclonal IgG and IgM antibodies. Antigen 'e' was detected by monoclonal blood grouping reagents anti-e (anti-RH5) antisera diluents cassette of Ortho BioVue System. The allele frequencies were calculated under the standard assumption of Hardy-Weinberg equilibrium, using the counting method of Cepellini et al. False positive and false negative results were strictly avoided by taking quality control measures at each step. An interpretation of both slide and tube tests were similar, if agglutination was observed then it was considered as presence of antigen or positive and if agglutination was not found then it indicates absence of antigen or negative. Peripheral drying and fibrin strands were voided while interpretation of results. Data was analyzed using statistical software R version 4.0.2 and Microsoft Excel

Result: In 1000 blood donors 971 were male and 29 were female. The mean age of donors was 29.69±6.47 years. Among study participants only 10 were positive with antigen'e'. Age ($p= 0.7288^{t}$) and gender ($p= 0.2644^{MC}$) were not significant with antigen 'e'.

Conclusion: In this study very less number of donors is positive with antigen 'e' which is contrast to the previous study findings.

Key words: Blood donors, Blood grouping and cross matching, Blood grouping antigens, Isoantigens

How to Cite

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INTRODUCTION

The blood grouping is more important while transfusion of blood from donors to recipient. Simultaneously, it is necessary to find other antigens which may develop antibodies in recipients. A patient with multiple transfusions may develop alloantibodies contrast to the uncommon Rhesus antigens (C, c, E and e).[1] The most common phenotype is DCCee and genotype is DCe/DCe (R1R1) in the western part of India.[2] There is a racial and genetic difference is observed in Rh antigen phenotype frequency in various population.[3,4]In Indian population the prevalence of RhD negative in lower than the European population and among ABO blood groups 'O' blood group is more prominent.[5,6] In Nigerian population the order of antigen observed is c > D > e > E > C.[7]

Identification of rare blood groups in routine screening of donors is quite essential to isolate antigen negative compatible blood to patients with significant alloantibodies.[8,9] Moreover, detection of alloantigen prior to the transfusion can prevent alloimmunisation in patient with multiple transfusion. The RBC phenotyping is aid in the development of phenotype database of blood donors.[10] There is a difference between serological and molecular interpretation of RhD assessment. In serological testing inconsistent result and partial RhD inference is observed with molecular testing.[11]

The gene RHCE gene codes for the antigens 'E' and 'e'. The 'E' and 'e' antigens are co-dominant and 'e' antigen is more recurrent than the 'E' antigen in all population.[11] This study aim and objective is to assess the antigen 'e' among blood group 'O' donors.

MATERIAL AND METHODS

Study design

The study was conducted from March 2018 to March 2019 in TTK Rotary blood bank, new Tippasandra, Banglore. Institutional ethical committee approval (EC No. PIEC/MLT/04/2022) and consent from donors were obtained. In this study we included 1000 'O' positive blood group donors, who were eligible as per the Drugs and Cosmetics Act 1940 and rules 1945. Inclusion criteria: donors with 'O' positive blood group, their age was between 18-60 years and weight was more than 50 kgs. Donor's hemoglobin level was ≥ 12 g% and pulse rate was 50 to 100 beats per minute. Blood pressure was measured with sphygmomanometer, diastolic pressure was between 50 to 100 mmHg and systolic pressure was 100 to 180 mmHg. Donor's body temperature was normal and oral temperature was 37.5^0 C. Exclusion criteria: Donors with history of cardiac arrest, hypertension, epilepsy, diabetes mellitus, malaria (within last three months) and kidney alignment. Women with a miscarriage for last six months were not included. Time period between consecutive blood donations should be three months and donor had been immunized for the last one month. Donors infected with Human Immunodeficiency Virus (HIV) and other viral infections. Donor must restrain from alcohol consumption for at least 24 hours before the blood donation.

Study procedure

Test principle: Human red cells with C, c, E and e antigens agglutinates with corresponding antibodies.

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Sample collection and processing: No prior preparation was required for the donors and sample was collected by approved techniques. Donors' blood sample was collected in 2 mL Ethylene diamine tetra acetic acid (EDTA) vials after blood donation. Initially ABO blood grouping and Rh (D) typing was done using Tulip diagnostics pvt.ltd Goa, India (monoclonal IgM antibody) and Span diagnostics pvt.ltd, Surat India (monoclonal IgM and IgG antibodies) respectively. Antigen 'e' was assessed using monoclonal blood grouping reagents anti-e (anti-RH5) antisera diluents cassette of Ortho BioVue System (Ortho clinical diagnostics, 1001 US Highway 202, Raritan, NJ 08869 USA), according to manufacturer's instructions.

A red cell antigen and phenotype frequencies of various blood group system was measured as

Number of donors positive for a particular antigen phenotype

Total number of donors screened

Results were expressed in percentage. The allele frequencies were calculated under the standard assumption of Hardy-Weinberg equilibrium, using the counting method of Cepellini et al.[12] False positive and false negative results were strictly avoided by taking quality control measures at each step. The red cell were tested for Rh group antigens using antisera D, C, E, c and e phenotype and it is presented in results using Wiener nomenclature.

Material required: Glass slides (60 X 85 mm), test tubes (12 X 75 mm), Pasteur pipettes. Isotonic saline, centrifuge, timer and mixer stick.

Procedure

Slide test: All the reagents were brought to room temperature before test procedure. A drop of ERYCLONE Anti 'e' reagent was placed on a clean glass slide. A single drop of whole blood was added to the reagent placed on slide and mixed it thoroughly over an area of 2.5 cm. Rock the slide with mixture gently back and forth. Observation was done for agglutination after 2 minutes interval.

Tube test: Red cell suspension of 5% was prepared with isotonic saline before test procedure. A drop of ERYCLONE Anti-e was added to designated test tube. Pipette a drop of red cell suspension in the test tube; incubate it at 37^0 C for five minutes and centrifuge for one minute at 1000 rpm (125g) or 20 seconds at 3400 rpm (1000 g).

Interpretation: An interpretation of both slide and tube tests were similar, if agglutination was observed then it was considered as presence of antigen or positive and if agglutination was not found then it indicates absence of antigen or negative. Peripheral drying and fibrin strands were voided while interpretation of results.

Data analysis

Data was analyzed using statistical software R version 4.0.2 and Microsoft Excel. Continuous variables were represented by mean and standard deviation and categorical variables were represented by frequency and percentage. To

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assess the association between categorical variables Chi-square test was applied. To compare mean over groups't-test was used. P-value less than or equal to 0.05 indicates statistical significance.

RESULT

The study included 1000 'O' Positive subjects, their mean age was 29.69±6.47years. In this study 971 males and 29 females were actively participated. Among study participants only 10 were positive with Ag 'e' (Table-1).

Table 1: Donors demographic data and Antigen 'e

Variables		**Number of subjects (%)**
Age (in years)	≤ 20	27 (2.7%)
	21-30	556 (55.6%)
	31-40	374 (37.4%)
	41-50	36 (3.6%)
	> 50	7 (0.7%)
Age (in years)		29.69±6.47
Gender	Female	29 (2.9%)
	Male	971 (97.1%)
Ag "e"	Negative	990 (99%)
	Positive	10 (1%)

Donors' age group (p=0.5177) and mean age (p=0.7288) .were not significant with antigen 'e'. Further gender (p= 0.2644) also not significant with antigen 'e'(Table-2).

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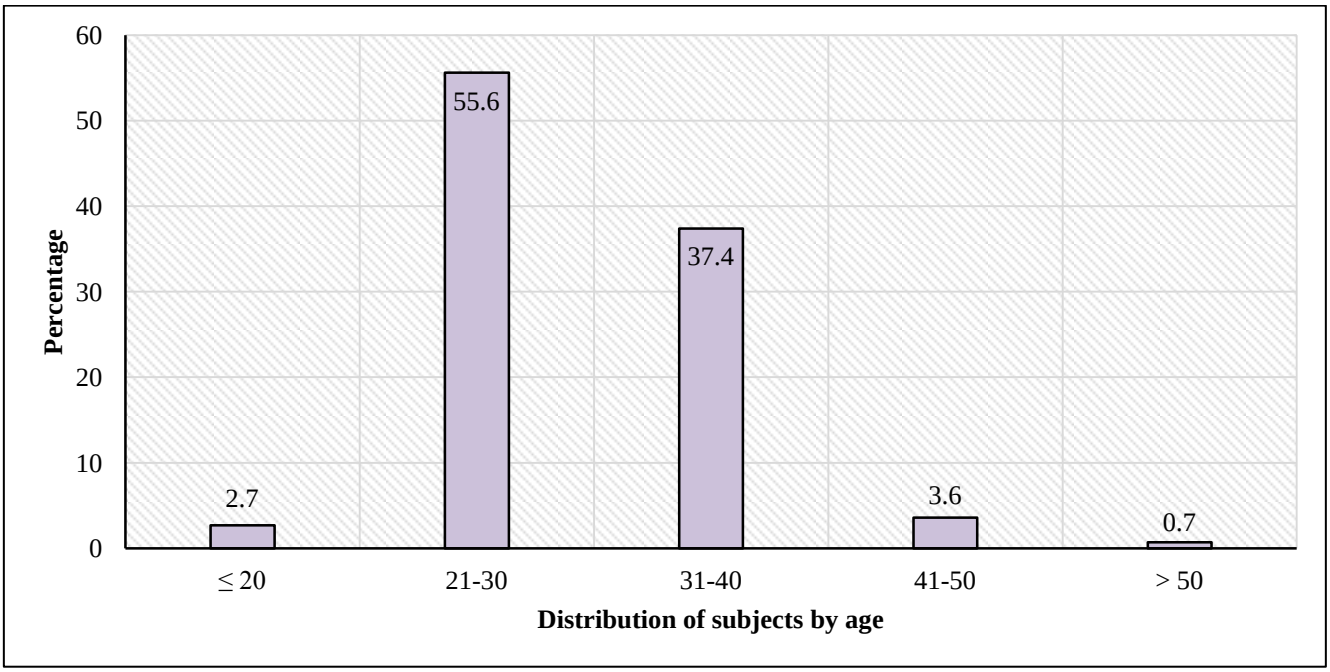

Figure 1: Distribution of subjects by age.

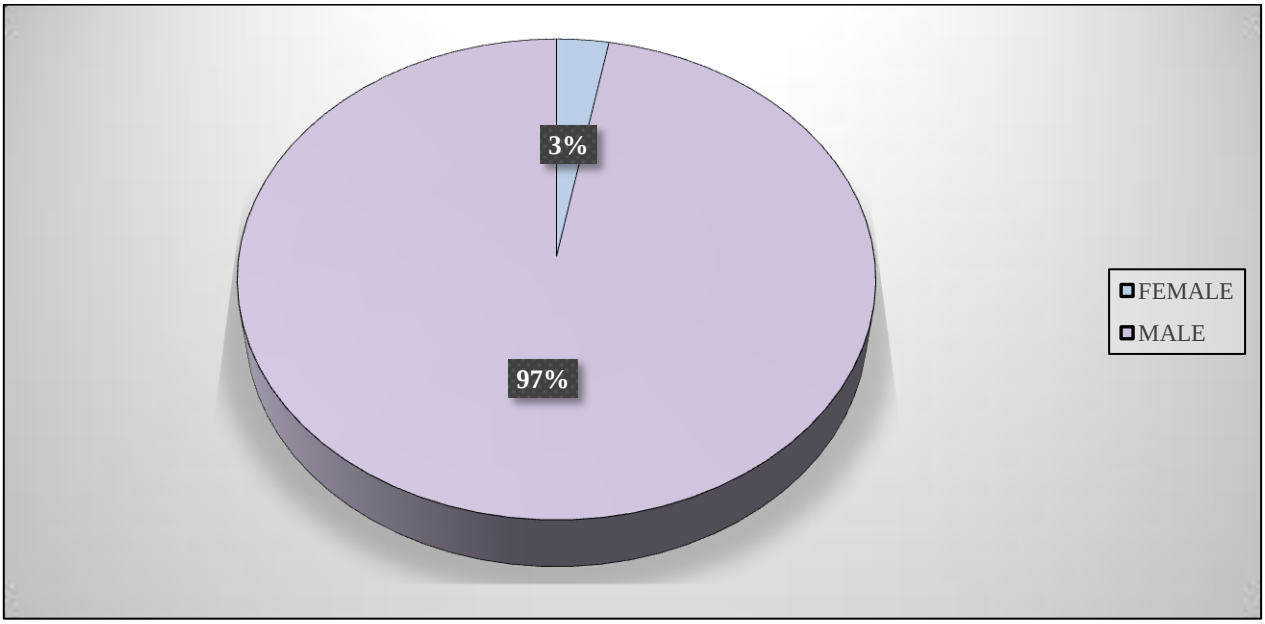

Figure 2: Distribution of subjects by gender.

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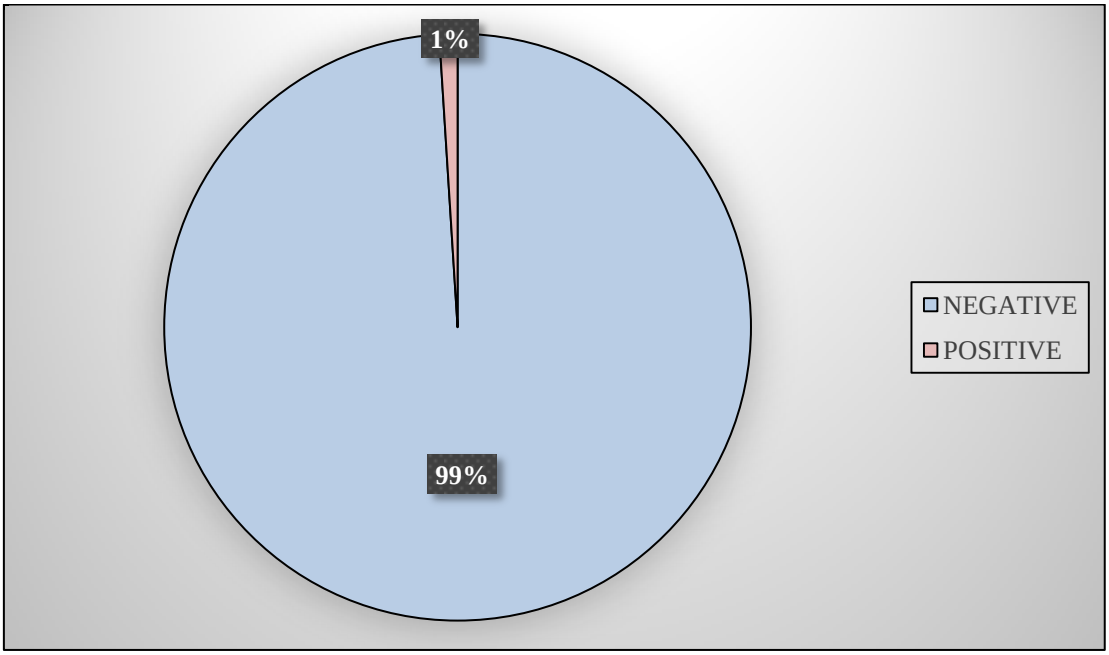

Figure 3: Distribution of subjects by Ag "e".

Below table compares the variables over Ag "e".

Table-2: Comparison of Ag 'e' with Demographic data of donors

Variables		Ag "e"		p-value
		Negative	Positive	
Age (in years)	≤ 20	27 (2.73%)	0 (0%)	0.5177[MC]
	21-30	552 (55.76%)	4 (40%)	
	31-40	368 (37.17%)	6 (60%)	
	41-50	36 (3.64%)	0 (0%)	
	> 50	7 (0.71%)	0 (0%)	
Age (in years)		29.69±6.48	30.4±5.52	0.7288[t]
Gender	Female	28 (2.83%)	1 (10%)	0.2644[MC]
	Male	962 (97.17%)	9 (90%)	

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Abbreviations: MC: Monte-Carlo's simulation used in Chi-square test, t: t-test.

From Chi-square test, there is no significant difference in the distribution of age and gender over Ag "e".

By two-sample t-test, there is no significant difference in the mean of age over Ag "e".

Below plots visualizes the above table.

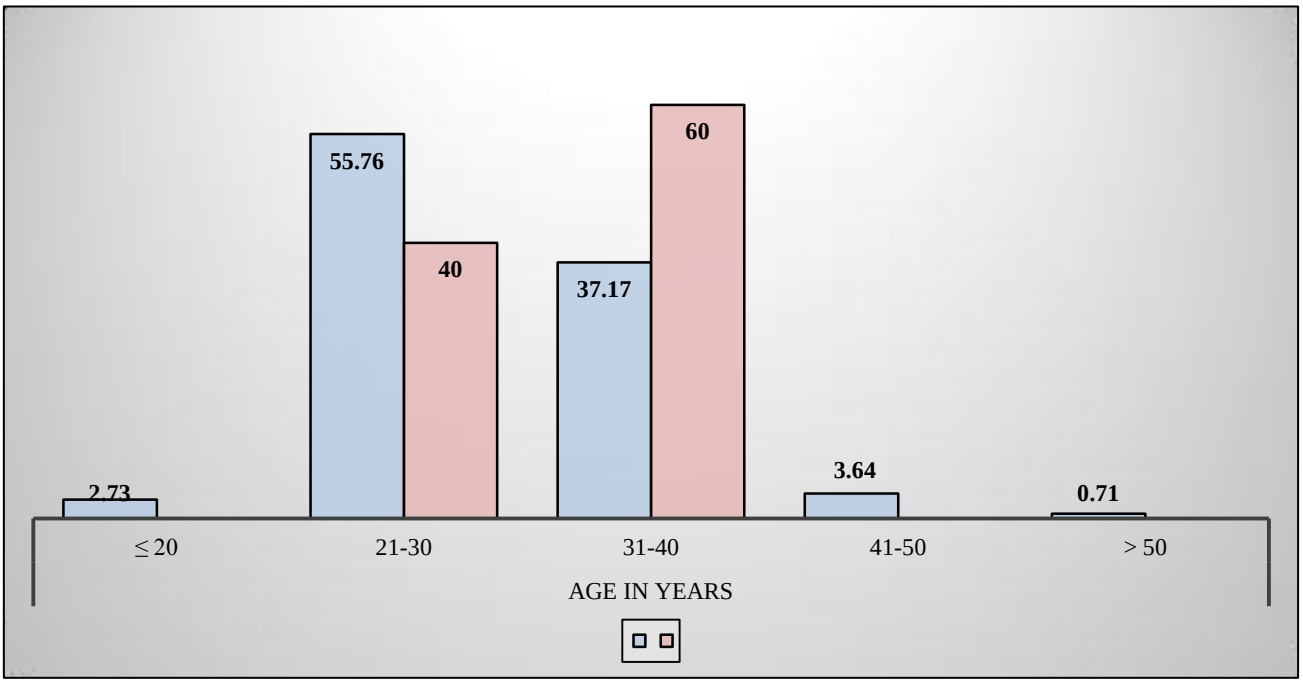

Figure 4: Distribution of subjects by age over Ag "e".

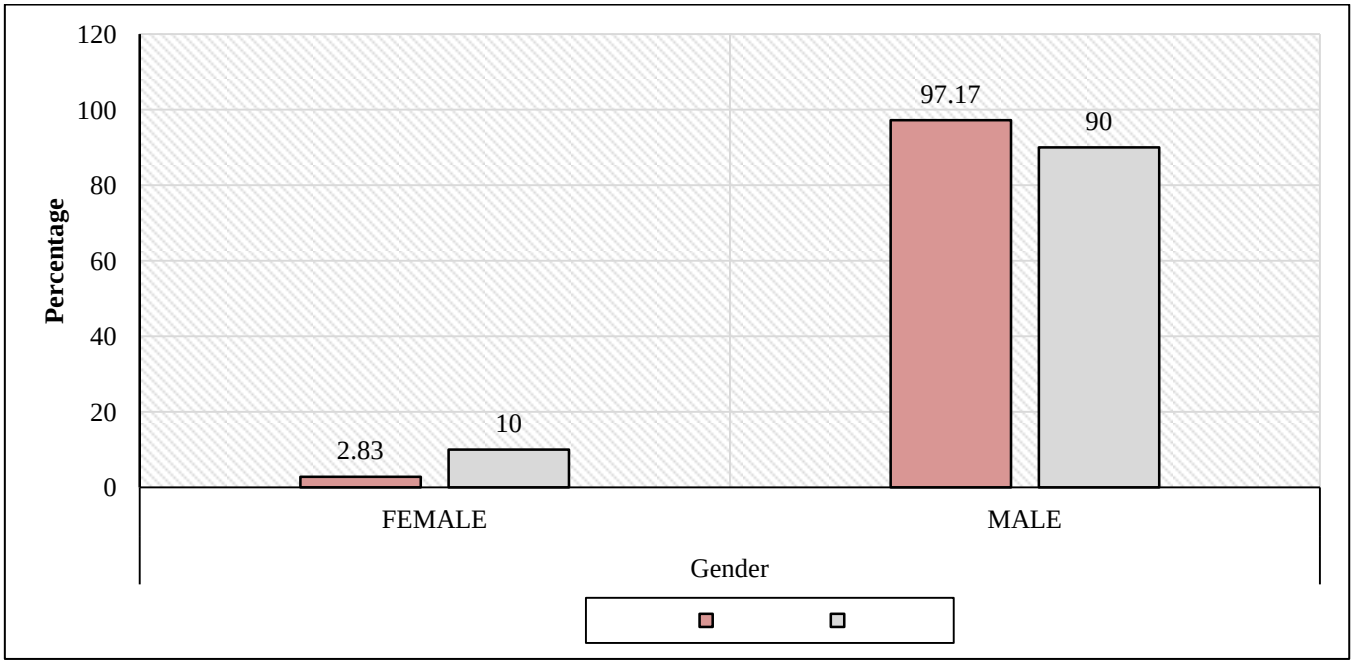

Figure 5: Distribution of subjects by gender over Ag "e".

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DISCUSSION

In this study we included 1000 'O' positive blood donors, their average age is 29.69±6.47 years and 971 (97.1%) are male and 29 (2.9%) are female donors. Among these donors only 10 (1%) donors are positive with antigen 'e'.

It is quite beneficial to determine minor blood group antigens such as C, c, E, e and K in a routine practice of blood grouping along with ABO and Rh(D) grouping prior to the first transfusion. That can prevent alloimmunisation in thalassaemia major recipients with multiple transfusions.[8] Among transfused alloimmunised patients, 3-4% is found with RBC alloimmunisation and antibodies are clinically significant.[13] In Sarkar et al and Garg et al studies, a number of participants included were higher than our study.[1,3] In their study they included all the blood donors, irrespective of their blood group. Whereas in our study only 'O' positive blood donors are included. An age of participants of Garg et al study was slightly higher than our study.[1] In Pachaury et al study, 'O' blood group donors with positive for phenotype antigen 'e' was much higher than our study findings.[2] Saleem et al study also finds higher percentage of phenotype antigen 'e' positive donors than our study.[8] In this study, after comparison of phenotype antigen 'e' with age and gender, none of them are significant. Determination of exact genotype is not possible without testing parents and other family members or by DNA testing. For this reason, most probable genotype is determined from gene frequency estimation.

Our study objective was to assess phenotype antigen 'e' among 'O' blood group donors, but it is observed that very less number of donors with phenotype antigen'e' positive. There is a large gap between our study results and previous studies with phenotype antigen'e' positive among O blood group donors. Hence, more research is needed to justify these two kinds of results.

CONCLUSION

In this study we observed that, there is a large gap between previous study findings and our study results with regards to antigen 'e' detection in the donors with blood group 'O'. Possibility of such result in this study could be the O blood group donors. Previous studies were incorporated a donors with irrespective of their blood group.

DECLARATION: I declare that this article has not been published elesewhere.

Conflicts of Interest: Nil

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