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Original Article

Prevalence and Haemolytic Significance of Red Cell Antibodies among Dangerous Universal Donors in a Tertiary Care Hospital in South India

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ABSTRACT

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Key words
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Background and Aims: A subgroup of group O individuals called 'dangerous universal donors' have immune (IgG) anti A and anti B antibodies which are active at 37˚C and capable of reacting with the red cells and causing lysis. The aim of this study was to find the prevalence of dangerous O group among the voluntary donor population and to assess the relation between the degree of haemolysis and the antibody titre.

Materials and Methods: Group O donors excepting those with history of transfusion or pregnancy were included in the study. The serum samples were tested for haemolysins as per standard procedure. The degree of haemolysis was graded and strongly haemolytic samples were further characterised for the type of immunoglobulin class after treatment with dithiothretiol. The results were coded and analysed using SPSS software.

Results: The age of the donors in this study ranged from 18 to 56 years. Majority were males. The prevalence of dangerous O group in our study population was found to be 10.75%. Within the dangerous O group samples, the titre of anti B IgG antibody was found to be higher than anti A IgG antibody. Titres for both anti A and anti B IgG antibodies ranged from 1:2 to 1:64.

Conclusions: A simple screening for donor haemolysins will help in identification of strongly haemolytic samples, which are likely to have high titres of IgG, particularly anti A antibody. This will prevent transfusion of blood containing high titres of immune anti A and anti B antibodies to non O group recipients.

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Introduction

The ABO blood group system is the most important blood group system for human blood transfusion. Austrian scientist Kar Landsteiner and his colleagues classified ABO blood group system into A, B, O, and AB depending on the presence or absence of blood group antigens on red cells [l]. The importance of a blood group system in clinical blood transfusion practice lies in the frequency of its antibodies which will destroy incompatible cells *in vivo* [2]. Almost everybody over the age of six months has clinically significant anti A or anti B antibodies in their serum when they lack corresponding antigens on their red cells [3].

The blood group O individual is called 'universal red cell donor' because his/her red cells possess no antigens which can be attacked by the naturally occurring anti A and anti B antibodies of recipient. The relative difficulty in getting ABO group identical blood sometimes necessitates the transfusion of group O donor blood to patients who are non-group O in emergency situations and for exchange transfusion in neonates born to non ABO group identical mothers, based on similar concept [4].

A subgroup of group O individuals have immune (IgG) anti A and anti B antibodies which are active at 37˚C and capable of reacting with the red cells of group A, B or AB recipients. These antibodies are called haemolysins [5]. Group O individuals having high titres of haemolysins are called 'dangerous universal donors' [6,7]. When plasma containing high titres of hemolytic anti A and anti B antibodies is transfused to non O group recipients, it can cause haemolysis of red cells of the recipients.

Our hospital is a tertiary care cancer hospital. Given the limited supply and short shelf-life of platelets and for optimal blood inventory management, it is a common practice to transfuse over the group platelets routinely in our hospital. The proportion of dangerous group O varies across populations, most studies report approximately 10-20% of group O donors belong to the dangerous O group [8-10], but such data from south Indian population is lacking. This study aims to find the prevalence of dangerous O group among the voluntary donor population at our centre and to assess the relation between the degree of haemolysis (*in vitro*) and the antibody titre.

Materials and Methods

The study protocol was approved by the Scientific Review Committee and of Regional Cancer Centre (IRB no 10/2015/18).

Voluntary group O donors attending our blood bank during the study period excepting those with history of transfusion or pregnancy were included in the study. The samples were collected after obtaining informed consent.

The study duration was 3 months. The sample size was calculated based on a study from north India, where prevalence of dangerous O group among donors was reported as 10-20%. Accordingly, 400 voluntary group O donors were recruited for the study.

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Blood group was confirmed by forward and reverse grouping. The serum samples were tested for haemolysins as per standard procedure (AABB technical manual). The degree of haemolysis was graded as follows: 3+ (complete haemolysis), 2+ (partial >50% but not complete), 1+ (trace haemolysis), and negative (no visible haemolysis). Serum samples having a score of 3+ and 2+ were considered strongly haemolytic and were further characterised for the type of immunoglobulin (Ig) class. For this, the serum was first treated with dithiothretiol and then titres of IgG anti A and anti B antibodies were estimated using indirect antiglobulin test. The result was recorded as the reciprocal phase of the highest serum dilution that showed macroscopic agglutination. Negative results were confirmed by addition of the Coomb's check cells to the negative tubes. A visual titre of more than 8 for anti A or anti B IgG antibody was taken as indicative of dangerous O group, based on the study by Oyedeji et al. [11].

Statistical analysis

The results were coded and entered in Microsoft Excel spreadsheet and analysed using SPSS software, Chicago, IL, USA version 11. Discrete variables were compared using Chi-square test. $p<0.05$ was considered as significant.

Results

The age of the donors in this study ranged from 18 to 56 years. Majority were males (92%). Out of 400 samples tested, strong haemolysis was seen in 60 samples (15%), out of which 3 were from females. Highest prevalence for haemolysins was seen in the 18-24 age groups (Fig. 1).

In the samples showing strong hemolysis, 88.3% was with A cells, 96.6% samples with B cells and 85% samples with both A and B cells. Titre of IgG antibody was estimated after dithiothretiol treatment using indirect antiglobulin test.

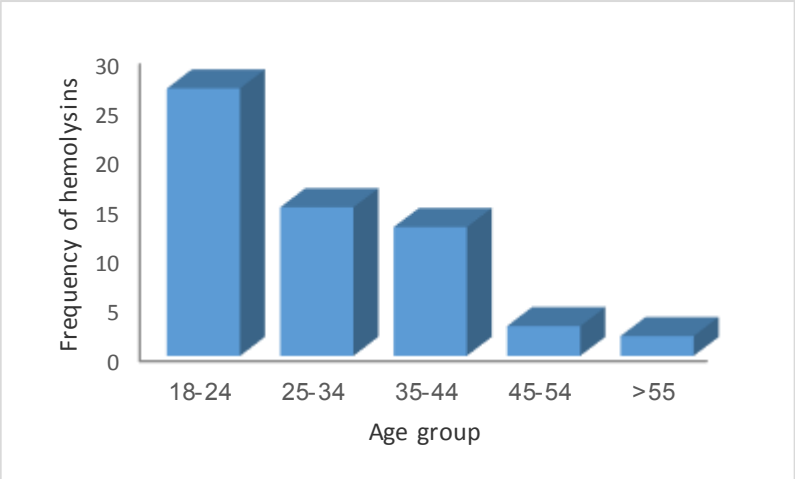

Fig. 1. Distribution of haemolysis positive samples across age group

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Titres for both anti A and anti B IgG antibodies ranged from 1:2 to 1:64. The highest frequency of antibody titre for both anti A and anti B IgG was 1:16 (Fig. 2). The mean titre of anti A IgG was 18.13 and anti B IgG antibody was 17.23. Samples having IgG antibody titre more than 8, with either A cells or B cells was taken as dangerous O group in this study. Accordingly, out of 60 samples which showed strong haemolysis, 43 samples had titre more than 8. Thus the prevalence of dangerous O group in our study population (out of 400) was found to be 10.75%. In samples showing grade 2 lysis with A cells, 48.8% samples showed anti A IgG antibody titre >8, while all samples with grade 3 haemolysis had antibody titre more than 8. Anti B IgG antibody titre more than 8 was seen in 46.3% samples showing grade 2 haemolysis with B cells, and all samples with grade 3 haemolysis had a titre more than 8. The p value was found significant for antibody titre more than 8 with grade 3 haemolysis. (Table 1).

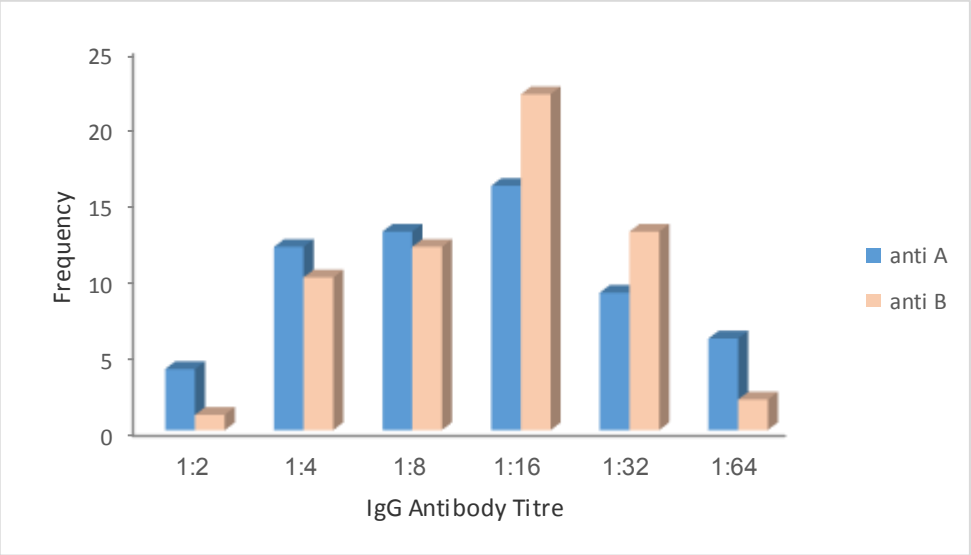

Fig. 2. Titres and frequency of anti A and anti B IgG antibodies

Table 1. Cross tabulation between grades of haemolysis with IgG antibody titre

Titre of Corresponding IgG Antibody	Hemolysis with A Cells		Hemolysis with B Cells	
	Grade 2	Grade 3	Grade 2	Grade 3
< 8	2 (51.2%)	0 (0%)	22 (53.7%)	0 (0%)
>8	21 (48.8%)	10 (100%)	19 (46.3%)	17 (100%)
P-value	0.003		0.000	

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Table 2. Cross tabulation between haemolysis with both A and B cells and IgG antibody titre

	Anti A IgG antibody titre		Anti B IgG antibody titre	
	<8	>8	<8	>8
Haemolysis with both A and B cells	21 (41.2%)	30 (58.8%)	18 (35.5%)	33 (64.7%)
P-value	0.008		0.249	

In samples showing strong hemolysis with both A and B cells, anti A IgG with titre more than 8 were seen in 58.8% and 64.7% with anti B IgG. The association between titre of anti A IgG antibody and strong hemolysis with both A and B cells was found stastically significant at p-value 0.008 (Table 2).

Discussion

15% of the present study population samples showed strong haemolysis when tested with corresponding cells. This is slightly lower compared to studies in African population, which had prevalence in the range 23.2% [12] to 55 [13-14] and comparable to study in Indian population [14]. The differences in prevalence can be due to variation in the serum-cell ratio used in the different studies. Geographical location has also been suggested as a possible reason for variations in prevalence obtained in various studies which found a higher frequency of anti A and anti B haemolysins in the Africans as compared to Asian population [6, 15-20]. This has been attributed to mosquito bites and parasitic infections of the gastrointestinal system [15].

In keeping with studies in literature, [19, 20] the present study also had a predominant male population. The prevalence of haemolysins was found to be higher in the younger age group. Saphire et al. [21] found no significant relation between age and prevalence of haemolysins, while study by Nancy et al. [22] found higher prevalence in 38-47 year age group.

Beta haemolysins were found to have a higher prevalence than alpha haemolysins in this study. This is in conformity to the findings of Olawumi et al. [12], Kagu et al. [13] and Thompson et al. [23]. While in the study by Oydeji et al. [11], beta haemolysins occurred less frequently than alpha haemolysins.

Titres for both anti A and anti B IgG antibodies ranged from 1:2 to 1:64. The highest frequency of antibody titre for both anti A and anti B was 1:16. In the study by Oyedeji et al. [11], titres for anti A haemolysins ranged from 1:2 to 1:32 while those for anti-B haemolysins ranged from 1:2 to 1:16.

In our study, the mean titre of anti A haemolysins was higher than that of anti B haemolysins. This result is consistent with the findings of Adewuyi et al. [24], Olawumi et al. [12] and Worlledge et al6, Grundbacher et al. [25] and Oyedeji et al. [11] who found higher titres for anti B haemolysins. The prevalence of dangerous O group (IgG titre>8) is 10.75% in this study population. 71.6% of the samples showing strong haemolysis belonged to the dangerous O group. Using similar criteria, Ugah et al. [14] found 62.65% of haemolysis positive samples to belong to dangerous O group. In a study from north western Nigeria, Uko et al. found 18.3% of haemolysis positive samples to

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have high titre haemolysins. They used a higher cut off titre of 64, which may be the reason for the lower prevalence of dangerous O group in their study. Studies from developed countries advocate an agglutinin titre of 128 or higher for identifying high titre units [20] using the column agglutination testing, which is more sensitive. Our study was based on conventional tube method. Maximum titre in our study obtained for both anti A and anti B IgG antibody was 64. In a study, Adeyemo [26] and Tisdall et al. [27] found maximum titre up to 256 for both IgG anti A and anti B antibodies. In this study, titres of more than 8 was seen in 7.7% and 9.2% of samples respectively for anti A and anti B IgG antibodies, while 5.7% samples had both anti A and anti B antibodies. Oyedeji et al. [11] found anti-A and anti B haemolysins with visual titre of 8 or higher in 10% and 8.6% of samples respectively. Olawumi et al. [12] found a prevalence titre of 2.0% for anti A and 2.8% for anti B and Kagu et al. [13] and a prevalence of 0.4% for anti A and 0.2% for anti-B. Such difference may be explained by the large sample size used by Kagu et al. [13] compared with what was used in our study.

Our study found a statistically significant association between grade of haemolysis and antibody titre. The higher the grade of haemolysis with A or B cells, the higher was the titre of the corresponding antibodies. Samples showing strong haemolysis with both A and B cells had statistically significant association with the titres of anti A IgG antibody.

Conclusion

Taking into consideration the findings of the present study, it is suggested that a simple screening for haemolysins in the serum of O group donors will help in identification of strongly haemolytic samples. This will avoid transfusion of plasma containing high titres of immune anti A and anti B antibodies to non O group recipients and prevent haemolytic transfusion reactions, which can be fatal.

Conflict of Interest

Authors have no affiliations with or involvement in any organization or entity with any financial interest such as educational grants; membership, employment, consultancies, or patent-licensing arrangements, or non-financial interest such as personal or professional relationships, affiliations, knowledge or beliefs in the subject matter or materials discussed in this manuscript.

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