



Full Length Research Paper

Presence of Atypical Antibodies in a Previously Transfused Population in Jos Metropolis

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Abstract

200 blood samples were collected from previously transfused participants who gave their informed consents (age between 5 – 60 years) after at least six months post transfusion, to determine the presence of atypical (immune) antibodies. The number of pint of blood received was also taken into consideration. 137 (68.5%) of the subjects had received 1 pint of blood, 22 (11.0%) of them had received 2 pint of blood and 41 (20.5%) had received more than 2 pint of blood. The antibody screening (at saline room temperature, saline 37°C, albumin and antihuman globulin) showed that the group that received 1 pint had 63.50% (87 samples out of the total of 137 samples), while recipients of 2 pint of blood had 68.18% (15 samples out of a total of 22 samples) and recipients of more than 2 pint of blood had 78.05% (32 samples out of a total of 41 samples). The cumulative prevalence is 68.0% (134 samples out of 200 samples). At $p < 0.05$ statistical level of significance, 5.81 was obtained, as against the tabulated value of 9.55. It was also observed that none of the samples reacted at room temperature. Furthermore, recipients of 2 pint and above reacted at saline 37°C; albumin and antihuman globulin, only 55.17% (48 samples out of 87 samples) reacted at saline 37°C and all of them reacted with albumin and antihuman globulin ($p < 0.05$). It is concluded that all the antibodies were immune, warm and incomplete in nature, confirming the presence of atypical antibodies in previously transfused persons.

Keywords: Blood, transfusion, antibodies, immune.

INTRODUCTION

Blood is a mixture of cells (red blood cells, white blood cells and platelets) and plasma among many other things; with diverse shapes, sizes and functions. The RBCs contain approximately 600 blood group antigens on its surface and they are grouped into at least 26 blood group system and subgroups based on molecular characteristics. These antigens and blood groups have been identified to be of great medical importance (Guyton and Hall, 2006). Plasma contains antibodies which could be immune in nature and they occur only in bodies that lack their corresponding antigen. Antigens may in certain circumstances react and cause harmful clinical effects like haemolytic disease of the newborn and other forms of blood transfusion reactions (Araujo *et al.*, 2002; Hoffbrand and Lewis, 1981; de Gruche, 1976; McDevitt and Benacerraf, 1969). Immune (incomplete) antibodies are produced in an individual as a result of stimulation by a blood group antigen which is not present on its RBCs or in its body fluid in a process called sensitization or immunization (Guyton and Hall, 2006; Leger, 2002). The

ability to produce such antibodies is referred to as immune response and once produced; such antibodies may remain in the body for up to many years (Zipursky and Israel, 1967). Immune antibodies characteristically react in warm temperature (37°C) in the presence of colloid or enhancement medium like bovine albumin, antihuman globulin and enzyme (Schonewille, 2006).

Blood and blood product transfusion, pregnancy and deliberate intravenous or intramuscular injections of specific antigen into a subject that lack such antigen have been identified as possible routes of initiating immunization (McDevitt and Benacerraf, 1969). Individuals who do not respond to stimulation at all are referred to as non-responders and there are several reasons for their inability to respond including genetic factors (McDevitt and Benacerraf, 1969). It has also been reported that the stimulation increases with increase in the number of administration of the antigenic substance. Blood transfusion in particular, is a very good means of initiating stimulation because of the large volume of

antibodies involved (Mollison *et al.*, 1979; de Gruche, 1976). The major manifestations of immune antibodies include blood transfusion reactions and hemolytic disease of the new born; at varying degrees and death may result (Guyton and Hall, 2006).

This study was carried out to determine the presence of atypical antibodies present in blood of select individuals who have had previous blood transfusions in our environment.

MATERIALS, SAMPLE COLLECTION AND METHODS

3 -5ml of anticoagulated whole blood were collected through venous puncture from a total of 200 previously transfused voluntary male subjects (of at least 6 months post transfusion) between the ages of 5 – 60 years, after obtaining ethical clearance and informed consent of the participants. The blood samples were allowed to clot for about 1 hour; the clots were then dislodged, spun for 5 minutes at 3000 RPM and the sera separated into plain containers. They were stored in a deep freezer (-20°C) for antibody Screening.

To be able to carry out the antibody screening, the following were prepared: -

- 1) Pooled blood group O cells (a pool of three different blood group O cells).
- 2) Positive (sensitized) and negative (unsensitized) blood group O positive cells. They were used as controls for antihuman globulin tests.

Preparation of pooled cells (using the method of Dacie and Lewis 1984)

Materials: - Test tubes, Bench Centrifuge, Pasture pipettes, Water bath

Reagent: - Normal saline (0.8%)

Samples: - 3 fresh whole blood group 'O'

Procedure

Equal volumes of 3 freshly collected whole blood group O were mixed together in a tube and washed 4 times using normal saline through spinning at 3000 RPM for 5 minutes and decanting the supernatant each time. At the end of the 4th washing, the deposit of cell mass was diluted to about 20% and used for antibody screening and antibody titration within 24 hours.

Preparation of positive and negative controls (using the method of Dacie and Lewis, 1984).

Procedure

Positive Control: - 10 drops of commercial anti – D were put in a tube, 2 drops of 50% saline suspension of washed red blood group O Rh positive red blood cells

was added and mixed properly. The mixture was incubated at 37°C in a water bath for 1 hour. It was washed 2 times and re-suspended to about 20% in saline for use.

Negative Control: - The same sample that was used for positive control washed 2 times using normal saline and re suspended to about 20% in normal saline for use (without any form of sensitization).

Antibody screening (using the method of Zupanska, 2003; Dacie and Lewis, 1984; Boorman *et al.*, 1977).

Procedure for each sample

100ml of test serum sample was put in a test tube. 1 drop of 20% washed normal saline suspension was added to it and mixed. It was incubated at 37°C in a water bath for 1 hour. The tube content was washed in normal saline 4 times by spinning at 3000RPM for 5 minutes and decanting the supernatant each time.

2 drops of antihuman globulin was gently added to the cell deposit, re-suspended for 1 minute and centrifuged lightly (150g) for 1 minute.

Tube content was examined macroscopically for agglutination and microscopically for negative and doubtful results. Results were recorded accordingly.

Positive and negative controls were treated in the same way at the same time with the test sample results and recorded accordingly. All the Positive samples were stored for antibody titration and identification.

Result key: C - Complete, V - Visual, +++ - Very large clumps, ++ - Large clumps, + - An even/distribution of clumps, W - Weak distribution of clumps, - - no agglutination.

Statistical analysis

Data processing and statistical analysis were performed with use of SPSS (Statistical Analysis for Social Science) and Microsoft excels software; mean, standard deviation and analysis of variance (ANOVA). A frequency distribution with percentages was used to show the trends within the data presentation as appropriate.

RESULTS

The sampling of each group is represented in table 1. A total of 200 samples were analyzed for the number of pints transfused. 137(68.5%) of the subject received 1 pint of blood, 22(11.0%) received 2 pints of blood and 41(20.5%) received more than two pints of blood out of the total of 200 samples collected. It shows that majority of the subjects received 1 pint of blood, followed by subject that received more than two pints.

Table 2 shows that majority of the subjects received

Table 1. Showing the Sample groupings

No. of Pint of Blood received by Group Members (Recipient)	No. of Blood samples received (Recipients)	Percentage (%)
1 pint	137	68.5
2Pints	22	11.0
>2 Pints	41	20.5
TOTAL	200	100

Table 2. Antibody screening

No. of Pint Of Blood received by Group Members (Recipients)	No. of blood samples Received (Recipients)	Reactivity for antibody screening		Non reactivity for antibody screening	
		No.	%	No.	%
1 pint	137 (100%)	87	63.50	50	36.50
2Pints	22 (100%)	15	68.18	7	31.82
>2 Pints	41 (100%)	32	78.05	9	22.00
TOTAL	200 (100%)	134	67.00	66	33.00

1 pint of blood, followed by subject that received more than two pints. The reaction expressed as a percentage of samples that were reactive for atypical antibody screening shows the negative ones and their corresponding percentage prevalence.

Table 3 expresses the antibody screening reactivity prevalence of subjects that received 1 pint, 2 pints and >2 pints in relation to the total antibody screening reactivity number (137) as 64.93%, 11.19% and 23.88% respectively.

Table 4 shows the trend of Conditions at which a Typical Antibodies Reacted. For 1 blood pint, Saline RT was 0%, Sal. 37°C was 48(55.17%), Alb. 37°C was 87(100%), and AHG was 87 (100%) while Typical Antibody Reacted of 2 blood pint was on Sal. RT was 0, Sal. 37°C was 13(86.67%), Alb. 37°C was 15(100%), AHG was 15(100%) and Typical Antibody Reacted of > 2 blood pint was on Sal. RT was 0, Sal. 37°C was 32(100%), Alb. 37°C was 32(100%), and AHG was 32 (100%). This means that all the antibodies reacted only at warm temperature and better with enhancement media like bovine albumin and antihuman globulin.

DISCUSSION

Two hundred (200) samples (participants) were analyzed for this study. They were grouped into three according to the number of pint of blood or transfusions received. The first group of 137 (68.5%) subjects is made up of those that received only 1 pint of blood, the second group is of 22 (11%) subjects made up of those that received 2 pint of blood and the third group of 41 (20.5%) is made up of those that received more than 2 pint of blood as indicated in table 4.1 and chart 4.1. 87 (63.50%) of the subjects that received 1 pint of blood (137) were positive for

atypical or immune antibodies, 15 (68.18%) of the subjects that received 2 pint of blood were positive while 32 (78.05%) were positive for atypical or immune antibodies. It also showed the negativity for antibody screening for the three groups. This means that some people were responders while others were non responders corroborating Ropper, (1999); Boorman and Dodd (1977). It also agrees with the position that blood/blood products are capable of eliciting the production of atypical or immune antibodies.

These results are higher than those recorded by Mollison, (1970), which is 50 - 60%. However, it should be noted that the volume used for their research were smaller than the volume used in our study. The high values used in this study is justifiable because volume is a factor in the determination of immunization (Jakobowicz *et al.*, 1972, Mollison 1979; Boorman and Dodd 1977; Hardisty and Weatherall 1974; Zipursky *et al.*, 1963). Another possible reason for the difference may be genetic or race since their data were Caucasian based (Mollison, 1979; McDevitt and Benaceraff, 1969). The present study was carried out on Negroes. Neville and Bryant, (1976) documented that antigenic strength is also responsible for atypical or immune antibody production but that cannot be verified by this study.

The increase in prevalence from those that received 1 pint, 2 pint and more that 2 pint of blood from 63.50%, 68.18% and 78.05% respectively show the importance of multiple stimulation in the production of atypical or immune antibodies as they serve as booster doses, eliciting a strong response and large amount of antibodies (Zupanska, 2003, Hoffbrand 1981; de Gruche 1976 and Zipursky *et al.*, 1963).

The progressive increase in the prevalence in relation to the number of blood transfusion is because the more the number of pint received, the higher the chances of

Table 3. Reactivity of antibody screening analysis

No. of Pint of Blood Received by Group Members (Recipients)	SAMPLES		REACTIVE	
	No. of Blood samples (Recipients)	Percentage (%)	No. reactive for typical antibody	Percentages reactivity for a typical antibody
1 pint	137	68.5	87	64.93
2Pints	22	11.0	15	11.19
>2 Pints	41	20.5	32	23.88
TOTAL	200	100	134	

Table 4. condition at which atypical antibodies reacted

No. of Pint of Blood Received by Group Members (Recipients)	Sal. RT	Sal. 37°C	Alb. 37°C	AHG
1 pint	0	48 (55.17%)	87(100%)	87(100%)
2 Pints	0	13(86.67%)	15(100%)	15(100%)
>2 Pints	0	32(100%)	32(100%)	32(100%)

Sal – saline, RT – room temperature, Alb – albumin and AHG – antihuman globulin

exposure to antigens that may be lacking in the recipient. However, multiple blood transfusions at short intervals do not serve as booster doses because they are administered almost at the same time.

However, 63.50% for receivers of 1 pint is high considering the clinical implication. Other reports of studies got 68.18% and 78.05% for receivers of 2 pint, and more than two pint respectively (Weiner and Waxler, 1948; Hardisty and Weatherall 1974; Rosenfield *et al.*, 1962). The means and standard deviations for those that received 1 pint, 2 pint and more than 2 pint are 68.50 ± 26.16 , 11.00 ± 5.66 and 20.50 ± 16.26 respectively, while the analysis of variance (ANOVA) in which the F-test (calculated value) is 5.81 at $p=0.05$ level of confidence compared to the tabulated value of 9.55. Hence, there is no significant difference between the antibody screening result and the number of pint received. Those sensitized in relation to the three groups (i.e. receivers of 1 pint, 2 pint and more than 2 pint of blood) out the total of 134, were positive for atypical or immune antibody screening. This reveals that the group of 1 pint had 64.9%, the group of 2 pint had 11.2% and the group of more than 2 pint had 23.9%. This is not of much clinical importance because it is dependent upon the sizes of the samples used in the different groups.

The findings reflect the magnitude of blood transfusion related production of atypical or immune antibodies. These clinical implications can be at varying degrees including death depending on the strength of the antibodies (Weiner and Waxler 1984; Hardisty and Weatherall 1974; Rosenfield *et al.*, 1962). This is an important thing to note in clinical practice particularly in taking blood transfusion related decisions.

It was observed that all the antibodies that were detected in the three groups were immune in nature because they were warm and incomplete antibodies

(none of them reacted at saline room temperature). More so, some reacted at saline 37°C and all reacted at albumin 37°C and antihuman globulin levels. This selective reactivity indicates the presence of a mixture of atypical or immune antibodies such as Rhesus (anti-D, anti-C, anti-E, anti-c and anti-e), Kell (K and k) Duffy and Kidd. (Dacie and Lewis 1984; Boorman and Dodd, 1977). The weaker the antibodies, the more the need for a stronger enhancement medium like bovine albumin, antihuman globulin and enzyme. It has been shown that receivers of 1 pint of blood had only 55.17% (48 samples out of the 87 that was analyzed), reacted at saline 37°C and receivers of 2 pints had 86.67% (13 samples out of the 15 samples that was analyzed). These correspond to the number of antibodies that were strong enough to react and the ones that were too weak to react at that condition. It is also an indication that there is mixture of antibodies in the samples. On the other hand, the reaction for the receivers of more than 2 pints justified the implication of multiple exposure booster effects. Similarly, all the samples in the three groups had 100% reactivity at albumin and antihuman globulin levels. These ones used enhancement media so it is also expected because of their high dielectric constant which dissipates the charge between red blood cells, thereby allowing closer contact as explained by Dacie and Lewis, (1984).

The statistical analysis showed that the means and standard deviations of the conditions of reactivity at saline 37°C, bovine albumin 37°C and antihuman globulin were 31.00 ± 17.52 , 44.67 ± 37.63 and 44.67 ± 37.64 respectively. At saline room temperature, both the mean and standard deviation are zero (0). The analysis of variance (ANOVA) in which the F-test (calculated value) is 1.70 at $p0.05$ level of confidence compare to the tabulated value of 4.07. Hence, there is no significant difference ($p>0.05$) between the various numbers of pints

of blood received by the subjects and the various conditions at which the atypical antibodies reacted, hence the H_0 is accepted.

LIMITATIONS

Several factors stood as limitations in the course of this study such as

- 1) Lack of adequate information about the clinical conditions that led to the blood transfusions.
- 2) Inability to identify the antibodies to know which blood groups to pay more attention to in our blood typing.
- 3) Lack of antigen and reagents to develop one.
- 4) High cost of reagents

CONCLUSION

The study has confirmed that blood transfusion is a major initiator for the stimulation of atypical or immune antibody production and established a stimulation prevalence of 63.50%, 68.18% and 78.05 % for the recipients of 1 pint, 2 pints and more than 2 pints of blood. It went further to show that multiple blood transfusion increases the prevalence of immunization and the titre. In addition, the study shows the production of multiple or mixed antibodies even in single blood transfusion and that this mixture increases in relation to the number of transfusion. This translates to increasing clinical implication with increase in the number of blood transfusion.

The study also confirmed that all the antibodies produced (immune antibodies) are incomplete and warm in nature (hence their non-reaction at saline room temperature). The fact that different antibodies have different strengths and conditions of reaction is also supported by this study as not all reacted at the various conditions of testing (saline 37°C, albumin and antihuman globulin).

Despite all the limitations, it is obvious that there is the need to expand our blood group typing beyond ABO and Rhesus for blood transfusion purposes. This will minimize the rate of atypical or immune antibody production. All detected atypical antibodies should be identified. This can be easily possible if the reagents, equipments and personnel are available in most if not all our health facilities. A more coordinated system need to be put in place in all health facilities with proper long lasting record system. Finally, all hands should be on deck to make the National Blood Transfusion Policy work.

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