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Racial difference between white and black Zimbabweans in the haemolytic activity of A, B, O antibodies

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Summary
Race is thought to be one of the factors determining the level of ABO antibodies. The objective of this study was to compare the haemolytic activity of anti-A and anti-B in two racial groups, Black and White Zimbabweans, living under similar conditions.

Serum haemolytic activity was assessed by comparing spectrophotometrically, released haemoglobin when known A1 and B red cells were incubated in equal volumes of subjects' serum and water.

Serum from Black subjects showed greater haemolytic activity of anti-A and anti-B than serum from White subjects. Within each racial group, anti-B showed greater haemolytic activity than anti-A.

Race may be an important factor in the haemolytic activity of ABO antibodies.

Keywords: *Haemolytic, anti-A, anti-B, white, black, Zimbabwe*

Résumé
La rate est cousidereé comme un des facteurs qui determine le tauc des anticorps ABO. L'objectiq de cette etude était de comparer l'activite hemolytique des Anti-A et Anti-B dans deux groupes raciaux, nouv et blanc Zimbawean vivant dans des condition similares.

L'activite hemolytique était évaluc éen comparant pan spectrophotometric, l'hemoglobine libérée lorsque des globules rouges d A1 et B connus était incubes a des volumes eqaux de serum et d'eau des sujets.

Le serum des sujets nous a moutre une plus grande activité hemodytique des anti-A et anti-B que lel serum des sujets blancs. Dans chaque groupe racial, anti B avant une plus grande activité hemoltique que anti-A.

La race serait un facteru important dans l'activite hemolytique des anticorps ABO.

Introduction

The serological characteristics of human antibodies including those of the ABO blood group system are known to be determined not only by physico-chemical properties of the proteins but also and to variable extent by age and sex of subjects; and genetic, racial and environmental factors (1,2). In an earlier communication (3), we showed that anti-A and anti-B agglutinin titres were greater in a high proportion of normal black Zimbabwean blood donors than reported for Caucasians. The present study was undertaken to quantify the haemolytic activity of anti-A and anti-B in both Zimbabwean Blacks and Whites and to reexamine the factor of race in the haemolytic characteristics of ABO antibodies.

Materials and methods

The study was carried out on blood donors at the National Blood Transfusion Service (NBTS) in Harare. The study subjects were all male, blood group O, Black and White Zimbabweans aged between 30 and 50 years, who had never been transfused. Males only were studied to avoid antibodies induced by pregnancy and a relatively narrow age band was selected to minimise differences due to age.

Anti-A and anti-B haemolysis was initiated by reacting subjects' fresh serum with known A1 and B red cells, respectively. The amounts of haemoglobin released by immune lysis in the test serum and the amounts released from an equal quantity of the same red cells by water lysis were then compared spectrophotometrically to quantify the haemolytic strength of the serum.

About four millilitres of serum were taken from each subject's clotted sample and kept at 4°C until processed. Haemolytic tests were carried out on the sera within 24 hours of blood collection to make full use of the subject's own serum complement which is required for red cell lysis. For each sample three tubes were set up for assessment of anti-A and three tubes for anti-B haemolytic activity.

The first, second and third tubes each contained 0.5ml of isotonic phosphate buffered saline (PBS), PH 7.4, 0.5ml of fresh donor's serum and 0.5ml of distilled water, respectively. Fresh group A1 and B red cells were washed thrice in saline and resuspended to 6% in PBS. To each of the first set of 3 tubes was added 0.2ml of 6% Al cells and to the second set 0.2ml of 6% B cells. The volume of neat red cells in 0.2ml of a 6% suspension is 0.012ml and when that volume is mixed with 0.5ml of test serum, the serum to cells ratio, by volume, comes to 41.7; or approximately 40 which is recommended for optional immune lysis reactions [4]. The first tube served as a zero-haemolysis negative control while the third tube provided 100% lysis.

A separate serum blank tube was set up for each sample which contained 0.5ml serum and 0.2ml of PBS. Another negative control tube was also set up in which 0.2ml of 6% suspension of known O-cells, which by definition contained no A or B antigens, was added to 0.5ml of subjects serum. All tubes were well mixed and incubated at 37°C for one hour and then centrifuged. Other tubes were set up in identical arrangement to the incubated ones and 2ml of PBS was placed in each tube. About 0.5ml of supernatant was carefully pipetted from each centrifuged tube into the corresponding tube in the second set with thorough mixing to produce a 1 in 5 dilution of each supernatant. The optical

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density of each suspension was measured in a spectrophotometer at 540nm using PBS as blank. The degree of lysis in each serum sample was calculated as recommended for the osmotic fragility test [5].

$$\text{Serum Haemolytic Activity (\%)} = \frac{\text{OD of serum lysis tube (serum + cells)} - \text{OD of serum blank (serum + PBS)}}{\text{OD of water lysis tube (water + cells)} - \text{OD of saline lysis tube (PBS + cells)}} \times 100$$

Any serum sample that showed visible lysis with O-cells was removed from the study and any batch of A or B cells that showed visible lysis with PBS only was not used for haemolytic tests. The serum samples were re-run after storage for one week at 4°C when the complement would have lost activity. Any haemolysis demonstrable at this stage was regarded as non-complement-mediated and non-specific and the sample was excluded from analysis. The results were analysed manually for means and standard deviations and the findings for Whites and Blacks were compared by Student's t-tests

Results

One hundred and seventy-six subjects were tested comprising 96 Blacks and 80 Whites. The haemolytic strength of anti-A and anti B in each subject's serum was expressed as the proportion (%) of A1 and B cells lysed. As shown in Table 1, statistically significant differences were found between the mean haemolytic activity of anti-A and anti-B of White and Black Zimbabwean men. The distribution of anti-A and anti-B haemolytic activities in Whites and Blacks is shown in Figure 1 and 2, respectively. Up to and greater than 50% anti-A haemolysis was found in 28.5% of Whites as compared to 49.0% of Blacks (P = 0.018). The corresponding values for anti-B haemolysis were 56.5% in Whites and 66.0% in Blacks (P = 0.032)

Table 1: Haemolytic activity of anti-A and anti-B in Black and White Zimbabwean men.

	ANTI-A			ANTI-B		
	Mean Haemolytic Activity %	'Range'	S-D	Mean Haemolytic Activity %	'Range'	S-D
Blacks (96)	48.4	2.8-100	29.8	62.8	4.0-100	28.0
Whites (80)	35.2	1.5-100	29.6	51.2	2.5-100	28.9
Total (176)	41.5	1.5-100		56.8	2.5-100	
Signific.	t=2.77	p=0.006		t=2.46	p=0.015	

S.D= Standard Deviation

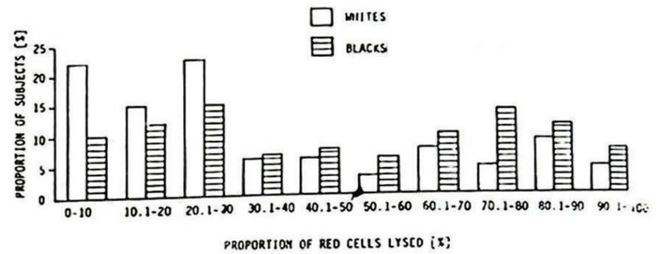

Fig. 1:

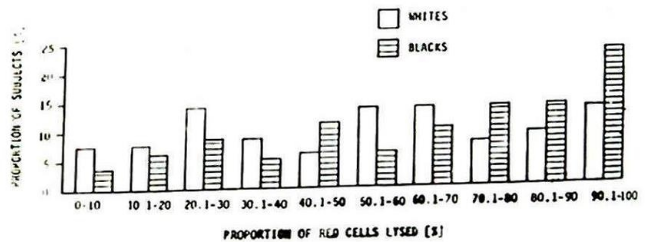

Fig. 2:

Discussion

Naturally occurring anti-A and anti-B antibodies are thought to arise in gestational life and early infancy by exposure to substances in the environment that have A- and B- antigen-like activity [6]. One of the factors that determine the level of antibody activity in individuals is thought to be the race [2]. In this study we have attempted to assess quantitatively the haemolytic activity of anti–A and anti-B in two racial groups in whom environmental and other non-racial factors, including lifestyle, are known to be equal. The findings confirm our earlier impression based on mere visual assessment of haemolysis [3] and the conclusions of Kulkarni *et al.* [7] that people of black origin produce more strongly haemolytic anti-A and anti-B than people of Caucasian descent. The mean haemolytic activities of anti-A and anti-B were significantly higher in Blacks than in Whites, though the range

Table 2: Haemolytic activity of anti-a and anti-b in black and white zimbabwean men; excluding subjects with greater than 95% haemolysis.

		ANTI-A		ANTI-B				
	No.	Mean heamolytic activity (%)	SD	Range(%)	No.	Mean heamolytic activity (%)	SD	Range(%)
BLACKS	81	46.35	22.6	2.8-91.5	74	59.02	25.1	4.0-94.7
WHITES	63	32.35	27.0	1.5-89.2	60	45.75	25.5	2.5-94.3
TOTAL	144	40.23		1.5-91.5	134	53.07		2.5-94.7
T-test (P-value)		*0.0025*				*0.004*		

of activity was similar in both groups. When samples with haemolytic activity greater than 95% which might technically fall outside the range of the test, were excluded from analysis, the differences between the means for Blacks and Whites were even more statistically significant (Table 2). It is conceivable that differences in complement activity in fresh serum samples, a factor which was not controlled in our study, could contribute to differences in haemolytic activity. However, in view of the greater anti-A and anti-B agglutinin titres in Blacks and theircorrelation with visual haemolysis as established in our earlier report [3], it is more probable that the differences in haemolytic activity found in the present study are due to differences in antibody activity. The difference was more marked for anti-A in which mean haemolytic activity was 1.4 times greater in Blacks than Whites. The corresponding Black to White mean Haemolytic activity ratio of anti –B was 1.2. These results are in agreement with those of Grunbacher [2] who found anti-A and anti-B agglutinin titres to be higher in Blacks than Whites and anti-B titres to be almost as high as anti-A in Blacks. Grunbacher's and our findings are in contrast to those of Redman who in a study of blood donors living in the United Kingdom, failed to show any significant difference between Blacks, Whites and Asians, in their IgM and IgG anti-A and anti-B levels [8]. It is however possible that agglutination and haemolysis tests; their demonstrable correlation notwithstanding [3]; do not measure exactly the same antibody activities. Further study is required to clarify this point.

Within each racial group we found anti-B haemolytic activity to be greater than anti-A. For Whites the anti-B to anti-A mean haemolytic activity ratio was 1.45 while for Blacks it was 1.3. This is consistent with a report in 1974 by Worlledge who found that in Nigerians haemolytic activity was more commonly anti-B than anti-A [9]. Similar results were obtained in another study of anti-A and anti-B haemolysins among pregnant and non-pregnant women, also in Nigeria [10]. Our results show that anti-B haemolytic activity was stronger than anti-A, not only in Blacks but also in Whites in Zimbabwe. This difference may therefore not be race-related and it may be that there is a stronger stimulation by B-antigen-like substances in the environment. On the other hand in our previous study of Black Zimbabweans, anti-A agglutinin titres were higher than anti-B [3]. A possible explanation of these findings may be that the A-antigen perhaps selectively induces agglutinins while the B antigen produces more of haemolysins. This hypothesis requires further elucidation.

Conclusion

The greater haemolytic activity of ABO antibodies in Blacks may have clinical implications. If it is true in Black women as it is in the men in our study it may partly account for the greater frequency and severity of ABO haemolytic disease of the newborn in Africans [11, 12]. Compared to Whites, the enhanced haemolytic activity of anti-A and anti-B in Blacks may produce more severe haemolytic reactions when Black recipients inadvertently receive ABO-incompatible red cells, or when plasma products from Black donors are erroneously administered to ABO incompatible recipients.

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