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Full Length Research Paper

The heterogeneity and distribution patterns of ABO and RH D phenotypes in the voluntary blood donors of Kenya

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Blood groups (antigens) are proteins, glycoproteins or glycolipids inherited surface markers on the red blood cell membranes, which determine the blood phenotypes of human beings. There are 36 blood group systems with over 300 antigens. Among them, ABO and Rh are of clinical significance. For a safe transfusion, a blood donor and recipient should be ABO and Rh D compatible. The heterogeneity of ABO and RhD blood groups systems shows variations in different parts of the world. In Kenya, there is limited study done on ABO and Rh D blood groups heterogeneity among blood donors. The aim of this study is to determine the heterogeneity and prevalence of ABO and Rh D blood groups among voluntary blood donors in Kenya, which is fundamental for compelling management of blood bank stocks. The study sites were Nairobi, Mombasa, Kisumu, Nakuru, Eldoret, Embu, Meru, Garrisa, Nyeri, Machakos, Thika, Voi, Malindi, Kericho, Kisii, Narok, Bungoma, Kitale, Lodwar, and Busia. The presence of blood groups was determined by serological techniques both microtitre and tube methods. Commercial monoclonal antisera (anti-A, anti-B, anti-D and Antihuman globulin) were used. Descriptive statistics and Chi-square were applied in data analysis and the results were presented in tables. The results showed that there was a statistical significance difference $p<0.01$ between the positive and negative blood types in both the ABO and Rh D systems. This study recommends an extended study with a large sample size and to include heterogeneity of other blood groups of clinical significance.

Key words: ABO, RHD, weak D, Microtitre, anti-human globulin (AHG) monoclonal antibodies, heterogeneity, prevalence.

INTRODUCTION

Blood groups (antigens) are inherited surface markers on the red blood cells membrane. These antigens are proteins, glycoproteins or glycolipids on the red cell membrane surface; they determine blood phenotypes of

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Table 1. A phenotype Heterogeneity.

Blood type	Number	Percentage
A	97	24.25
Total	400	100

Table 2. A phenotype variation across the ABO phenotypes.

Parameter	A phenotype	
	N	Percentage
Total	97	100.0
Variation	2.87	100.0

human beings.

Blood groups systems consist of single or more antigens that are governed by a solitary gene locus or by homologous genes (Dean 2005; Daniel, 2004). They were first discovered in 1901 by Karl Landsteiner (Nobel Prize Winner) when he saw that plasma from a few people agglutinated red cells from others but failed to agglutinate others thus he named the blood as A, B and O. Afterwards, Decastello and Sturli added a fourth group AB (Landsteiner, 1931; Daniels, 2004). Forty years later, Levine and Stetson depicted the Rh blood amass framework. This was while they were investigating a Haemolytic Transfusion Reaction (HTR) in a woman who reacted to a blood transfusion from her husband (Scott, 2004).

ABO and Rh blood group systems are of clinically significance in transfusion medicine because they can cause HTRs and haemolytic disease of the fetus and the newborn plus transplant rejection (Daniels and Bromilow, 2010). By 2016, up to 346 antigens have been described of which 306 are clustered within 36 blood group systems. The remaining 38 antigens are yet to be assigned to any known blood group systems (Story et al., 2016).

Before 1950s, human blood groups were known as pattern of inheritance that could only be detected serologically. In the 1950s, biochemical analysis aided the revelation of the structure of carbohydrate and protein antigen, which resulted in the description of the functions of some blood group antigens. Some of these surface markers are now known to have specific functions such as membrane transporters seen in Diego (anion transporter), Kidd (urea transporter), and the Colton (water channel) receptors expressed by Duffy, complement regulatory glycoproteins seen in Cromer and Knops, enzymatic function as observed with MN antigen. Functions of the Rh proteins and the Rh-associated glycoproteins RhAG proteins are not yet fully understood, but many assumptions have been made due to their structural characteristics of membrane transporters suggesting their involvement in ammonium transport or in maintaining the symmetry of the phospholipid in the red cell membrane. Many suggestions have been made to indicate that the Rh proteins are involved in CO_2 and O_2 transportation. However, all these assumptions are un-confirmed (Daniel, 2004; Daniel and Bromilow, 2010).

Most of the 36 defined blood groups systems exhibit the null phenotype, which is associated with lack of specific blood group antigen expression on the red cell membranes. The null phenotype is detected serologically by absence of agglutination with specific antisera. Molecular analysis has revealed that the null phenotypes is caused by mutations, including deletions, missense mutations, frame shift mutations, introns splice-site and promoter mutations which cause lack of antigen expression on the surface of the red cell. The null phenotypes are rare but clinically significant. Individuals with null phenotypes are at risk of immunization due to transfusion of incompatible blood or pregnancy via fetal maternal haemorrhage. Variation of antigen expression has also been reported which is caused by nucleotide duplications and hybrid gene as seen in weak or/and partial RhD (Daniels and Bromilow, 2010; Wagner et al., 2000, 2002).

MATERIALS AND METHODS

ABO and RhD typing

A total of 400 anonymous left over blood samples collected in EDTA vacutainers were randomly sampled from 20 registered KNBTS sites. The whole blood samples were typed serologically by use of commercially acquired monoclonal antisera at national blood grouping laboratory at KNBTS. Both microtitre and tube grouping methods were employed. Forward typing of the samples was done in the microtitre plate by mixing 1 drop of 2% red blood cells saline suspension that was prepared in normal saline with 1 drop of the appropriate antisera (anti-A and anti-B).

Reverse grouping was done using the microtitre plate method by mixing 1 drop of plasma of the whole blood samples with 1 drop of known A,B, and O red blood cells. Rh typing was accomplished by mixing I drop of 3% red cell saline suspension with 1 drop of anti-D in the microtitre plates. All the microtitre plates and contents were loaded into the microtitre plate centrifuge and spun at 2000 rpm for 1 min. The microtitre plates' contents were locked for 1 min and examined using a magnifying glass mirror for agglutination. The Rh D negative blood samples were retyped using the tube method and confirmed for D^u positive using indirect antihuman globulin test tube method.

RESULTS

The results showed that there was a statistically significant difference $p < 0.01$ between the positive and negative blood types in both the ABO and Rh D systems. Blood group A (24.25%): positive (23.25%), negative (0.5%), weak D (0.25%) are shown in Tables 1 to 3; Blood group B (18.75%): positive (17.25%), negative (1%), weak D (0.25%) are shown in Tables 4 to 6;

Table 3. Differences between A Phenotype categories.

ABO blood group system		A blood group category		Total
		A Positive	A Negative	
Positive	N	95	0	95
	%	100	0	97.94
Negative	N	0	2	2
	%	0	100	2.06
Total	N	95	2	97
	%	100	100	100

Pearson Chi^2 (1) = 97.0000; Pr < 0.001.

Table 4. Blood group B variation across the ABO Blood groups.

Parameter	B	
	Number	Percentage
Total	74	100.0
Variation	5.38	100.0

Table 5. Blood group B in percentages.

Blood type	Number	Percentage
B	74	18.5
Total	400	100

Table 6. Blood type B categories variations.

ABO blood group system		B blood group Categories		Total
		B Positive	B Negative	
Positive	N	70	0	70
	%	100	0	94.59
Negative	N	0	4	4
	%	0	100	5.41
Total	N	70	4	74
	%	100	100	100

Pearson Chi^2 (1) = 94.0000; Pr < 0.001.

Blood group O (51.75%): positive (46.75%), negative (3.5%), weak D (1.5%) are shown in Tables 7 to 9. The least was AB (5.5%) and all were positive (Tables 10 and 11). Among the RhD system, D positive was the most common (93%), followed by D negative (5%) and weak D was the least common (2%) (Tables 12 and 13).

DISCUSSION

Knowledge of blood group ABO heterogeneity is very important in planning for recruitment of voluntary blood donors in a country. It is also essential in the management of blood stocks, be it in the transfusing

Table 7. Frequency of blood group O in percentages.

Blood type	Number	Percentage
O	207	51.75
Total	400	100

Table 8. Blood group O variation.

Parameter	Blood group O	
	Number	Percentage
Total	207	100.0
Variation	2.98	100.0

Table 9. Blood group O categories differences.

ABO blood group system		Blood group O categories		Total
		O Positive	O Negative	
Positive	N	193	0	193
	%	100	0	93.24
Negative	N	0	14	14
	%	0	100	6.76
Total	N	193	14	207
	%	100	100	100

Pearson Chi^2 (1) = 207.0000; Pr < 0.001.

Table 10. Blood group AB distribution in percentages.

Blood type	Number	Percentage
AB	22	5.5
Total	400	100

Table 11. AB phenotype variation.

Parameter	AB	
	N	Percentage
Total	22	100.0
Variation	0.73	100.0

facilities or the national blood service activities. Typing donors and recipients blood for ABO antigens is essential in blood transfusion, genetic research, organ transplantation, settling paternity disputes, and in cases of immunization complications (Ahuja et al., 2015; Neil, 2006).

The finding of this study analysis revealed that A phenotype heterogeneity was 97 (24.25%) in the donor population. Further, the analysis of the 97 samples indicated a variation of 2.87 across the ABO blood groups. There was a statistically significant difference ($p<0.001$) between the A positive and A negative blood

Table 12. RhD blood group variation.

RHD	Number	%
RHD Positive	372	93
RHD Negative	20	5
Weak D	8	2
Total	400	100

Table 13. RhD categories variation.

RhD positive	RhD Negative				Total
	Zero	One	Two	Three	
16	0	0	0	1	1
17	0	0	1	1	2
18	0	3	2	0	5
19	3	5	0	0	8
20	4	0	0	0	4
Total	7	8	3	2	20

Pearson Chi^2 (1) = 30.6220; Pr = 0.002.

phenotypes in the ABO system. The outcome of other studies from different countries have also shown a wide difference in the A phenotype heterogeneity; Kurd 37.2% (positive 29.99% and negative 2.75%). In Ethiopia, prevalence is 32.74% (Alemu and Mama, 2016; Jaff, 2010). Different patterns of A phenotype have also been observed in other parts of the world (Agrawal et al., 2014; Ahuja et al., 2015; Periyavan et al., 2010). In Delhi India, the A phenotype heterogeneity was recorded as 24.66% (Ahuja et al., 2015). The Delhi outcome was almost similar to the findings of this study of 24.25%. The frequency of the A phenotype in UK was recorded as 43% of which A positive was 36.6% and A negative was 6.5%, respectively (Learoyd et al., 2009; Daniels and Bromilow, 2010). This is to mean that in the ABO system, individuals who are of A phenotype are either A positive or A negative.

The study outcome revealed that the variation of blood group A in the Kenyan donor population is 18.5% with a variation of 5.38 across the ABO blood groups. These results were further categorized into two; B positive which showed 17.5 and 1% B negative. There was a statistically significant difference $p<0.001$ between the B positive and B negative categories in the ABO blood group system.

According to studies from other countries, wide variations were also recorded in the B phenotype. In UK, blood group B was recorded as 9%. B positive was recorded as 7.6% and negative as 1.4% (Learoyd et al., 2009). In Ethiopia, record showed that B type was 20.9% (Alemu and Mama, 2016). Among the Kurd population, B blood group was 23.84% (Jaff, 2010). India recorded 32.26% of blood B type (Agrawal et al., 2014), 36.4% was recorded in Western Rajasthan (Rajshree and Raj, 2013), 34.15% in Bangladesh (Behra and Joshi, 2013), 32.73% in Delhi (Ahuja et al., 2015) and 29.95% in Bangalore (Periyavan et al., 2010). This was also shown by studies carried out in UK, Ethiopia, and Kurd (Learoyd et al., 2009; Daniel et al., 2010; Alemu and Mama, 2016; Jaff, 2010). Studies carried out in India showed that blood group B was the most common in that population (Agrawal et al., 2014; Behra and Joshi, 2013; Rajshree and Raj, 2013; Neil, 2006; Ahuja et al., 2015; Periyavan et al., 2010).

The study outcome revealed that the frequency of blood group O was 51.75% (207/400) with a variation of 2.98 across the ABO blood groups. This outcome was further separated into O positive and O negative categories. The O positive was 193 (48.25%) and negative was 14 (3.5%). The outcome also indicated that there was a statistically significant difference $p<0.001$ between the O positive and negative categories in the ABO blood system. The results showed a slight variation in the blood group O frequency when compared with other studies. Studies from other countries such as UK, Kurd, and Ethiopia also showed different frequency patterns of blood group O. In UK, the frequency of phenotype O was 45%. Among the 45% O blood type, O positive was shown to be 38.25% and negative 6.75% (Learoyd et al., 2009; Daniel and Bromilow, 2010). In Kurd O phenotype frequency was 37.16% of which O positive was 34.03% and O negative was 3.13% (Jaff, 2010). Studies from India showed that O blood group frequency was 37.1% (Agrawal et al., 2014). The frequency of O phenotype

among the Ethiopians was 42.1% (Alemu and Mama, 2016) and 30.42% in Delhi (Ahuja et al., 2015). The study results revealed that blood group O was the most common blood phenotype in Kenya. This compares well with other studies carried out in UK, Ethiopia and Kurd. In summary, blood group O frequency was 51.75% of which 48.25% was O positive and 3.5% was negative. The variation was 2.98 across the ABO blood groups. There was a statistically significant difference $p<0.001$ between the positive and negative categories.

The study outcome indicated that AB type was 22 (5.5%) with a variation of 0.73 among the ABO blood groups. The study results grouped all the AB blood type in the AB positive category. Most studies carried out in different countries also recorded variation in the distribution of AB blood group. In UK, AB phenotype distribution was shown to be 3%, AB positive was 2.5% and negative was 0.5% (Learoyd et al., 2009). Study outcome from Kurds indicated that AB was 6.35% in the donor population. Among Kurd population, for AB distribution pattern, 6.02% was AB positive and 0.33% was negative (Jaff, 2010). In an Ethiopian study, AB distribution pattern was 4.3% (Alemu and Mama, 2016). In the Indian population, the AB blood phenotype distribution pattern ranges from 6.3% in Western Rajasthan (Rajshree and Raj, 2013) to 12.2% in Delhi (Ahuja et al., 2015)

The study revealed that there was variation in the RhD system. RhD positive was 93%, negative was 5% and weak D was 2%. There was a statistically significant difference $p<0.001$ between the D positive and D negative. Studies from UK indicated that 85% of the population was D positive and 15% D negative (Learoyd et al., 2009). In Ethiopia, 92.8% was of D positive and 7.3% was negative (Alemu and Mama, 2016).

Study from Albania indicated that the Rh D positive was 89.0%, 10.86% was negative and 0.14% was weak D (Xhetani et al., 2014). In Indian, D antigen variation was shown to range from 91.7 to 94.61% (Rajshree and Raj, 2013; Agrawal et al., 2014).

Conclusion

The study revealed that there is heterogeneity in the ABO and RhD blood groups.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

RECOMMENDATION

There is need to expand this study to incorporate more samples and also to determine if the heterogeneity exhibited by the ABO and RhD phenotypes exists in other blood group antigens that may be of clinical significance.

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ABBREVIATIONS

AHG, Anti human globulin; **Du**, weakened form of Rh D antigen; **ICT,** indirect Coombs test; **EDTA,** ethylenediaminetetraacetic acid; **G/MLS,** grams per deciliters; **HDFN,** haemolytic disease of the fetus and the new born**; HTR,** haemolytic transfusion reaction; **KNBTS,** Kenya National Blood Transfusion Service; **RBCs,** red blood cells.

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