

# Association of Some Haemoglobin Variants with ABO and Rhesus D Antigen Phenotype in Kano Metropolis

Najib Isyaku Ali, Garba Nura\*

Department of Medical Laboratory Science, Bayero University, Kano, Nigeria

## Abstract

Haemoglobin (Hb) variants are mutant form of haemoglobin caused by genetic variations. Several researches revealed that some diseases are associated with some specific blood groups. The aim of this research was to determine the association between some haemoglobin variants and the ABO and Rhesus D antigen Phenotype in Kano Metropolis. A total of 120 participants with different haemoglobin variants were recruited in this study for the period of eight months, April through November, 2016. Forty (40) apparently healthy subjects who had normal haemoglobin AA were used as controls. Hb phenotype was confirmed by Hb electrophoresis at an alkaline PH. While ABO and Rhesus D antigen phenotypes were determined by tile and tube methods using antisera from Lorne Laboratories, UK. Blood group O was found to be higher in those with haemoglobin SS and SC, and blood group B was found to be higher in those with Hb AC. In Hb SS, blood group O (53.33%) predominates followed by A (18.33%), B (16.67%) and AB (11.67%). In Hb SC, blood group O (56.6%) predominates followed by group B (20%), AB (16.67%) and A (6.67%). But in Hb AC, blood group B (40%) predominates followed by O (30%), A (16.67%) and AB (13.33%). Statistically negative association was established between haemoglobin variants (Hb SS, Hb SC and Hb AC) and the ABO and Rhesus D antigen Phenotypes in this study. ABO and Rhesus D antigens may not be associated with the inheritance of haemoglobinSS, haemoglobinSC and haemoglobinAC.

## Keywords

Haemoglobin Variants, Kano Metropolis, ABO and Rhesus D Antigen

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## 1. Introduction

Haemoglobin is the oxygen carrying pigment of the red blood cells [1]. It is a chromoprotein which contains four polypeptide chain, and haeme groups. Haemoglobin genotypes are inherited characters determined by different combination of these chains. Defects in its genes can produce abnormal haemoglobin which leads to conditions known as haemoglobinopathies [1]. The normal haemoglobin and the most occurring referred to as haemoglobin A (HbA) genotype and other abnormal ones like haemoglobin S (HbS), which is a variant form of haemoglobin [2]. HbS differs from HbA by substitution of

valine at position six of the  $\beta$ -chain with glutamine. HbC differs from HbA by substituting lysine, at position 6 of the  $\beta$ -chain with glutamine. This variant causes a mild chronic hemolytic anaemia. The homozygous haemoglobin of most individuals is HbAA (normal), other variants include HbAS (sickle cell trait), HbAC, HbSS (sickle cell disease), HbSC etc. These variants cause moderate to severe hemolytic anemia leading to high degree of morbidity and mortality [3, 4]. Although heterozygotes are symptoms free, they present specific hematological characteristics that are useful for their identification [5]. The World Health Organization (WHO) figures estimate that 6% of the world population is a carrier for Haemoglobin disorders [4]. The

\* Corresponding author

E-mail address: garbanura001@gmail.com (G. Nura)

highest prevalence of sickle cell disorder is found among people of African or Caribbean descent and may occur among those from the Eastern Mediterranean, Middle East, India and Pakistan [5].

Blood groups are groups of antigens located on the surface of the red cell membrane. Although about 400 blood grouping antigens have been reported, ABO and Rhesus (Rh) being the 1st and 4th to be discovered respectively are the most frequently studied genetic markers in humans [6]. Apart from their importance in blood transfusion practice, they are useful in genetic studies of populations and also resolving medico-legal issues like disputed parentage [6].

Several studies reported an association between ABO and Rhesus blood groups with other diseases; for example a higher prevalence of stomach cancer was reported among people with blood group A [3], greater susceptibility of group O individuals to peptic ulcers, non-O blood group had an 11% increased risk of developing Coronary Heart Disease compared to O blood group individuals [7], pernicious anaemia has been reported more among group A than group O individuals, group O persons are more likely to develop duodenal ulcers than A, B, or AB blood groups, prostate cancer and rectal cancer appear to be associated with A and B blood groups respectively [8].

The result of this study will therefore form the basis for providing genetic counseling services to people on matters that concern crucial decisions like marriage and blood transfusion which will help to reduce haemoglobinopathies and hemolytic diseases respectively.

The aim of this research was to determine the association of some haemoglobin variants with ABO and Rhesus D antigen phenotype in Kano metropolis.

## 2. Methods

### 2.1. Study Area

This study was conducted at three (3) different hospitals, Aminu Kano Teaching Hospital, Murtala Muhammad Specialist Hospital and Abdullahi Wase Specialist Hospital Nassarawa. The 3 Hospitals are situated within Kano Metropolis. Kano state is a state located in the North-western Nigeria [9]. It lies between latitude 11°30'N and longitude 8°30'E. It was created on May 27, 1967 from part of the Northern Region. Kano state borders Katsina to the north-west, Jigawa state to the north-east, Bauchi state to the south-east and Kaduna state to the south-west. The total land area of Kano state is 20,760sq kilometer with a population of 9,383,682 based on the official 2006 National Population and Housing Census [9].

### 2.2. Study Design

This study is a prospective cross sectional study design

### 2.3. Study Population

The study comprises 120 individuals with Hb pattern of SS, SC and AC attending Aminu Kano Teaching Hospital, Murtala Muhammad Specialist Hospital and Abdullahi Wase Specialist Hospital Nassarawa.

### 2.4. Ethical Approval

An ethical approval to carry out the research was sought from Research Ethical Committee of Aminu Kano Teaching Hospital (AKTH) and Ministry of health, Kano.

#### 2.4.1. Informed Consent

A written informed consent was obtained from all subjects before inclusion using an approved protocol.

#### 2.4.2. Inclusion Criteria

Only on individuals confirmed with Hb pattern of SS, SC and AC were included in the study. Apparently healthy individuals with haemoglobin AA were also recruited as controls.

#### 2.4.3. Exclusion Criteria

Individuals with other Hb pattern like AS and CC were not recruited and considered in this study.

### 2.5. Sample Collection and Processing

Using a disposable needle and syringe, 2ml of blood was collected from each subject by venipuncture and transferred into a labeled ethylenediamine tetra acetic acid (EDTA) containing bottle.

#### 2.5.1. Haemoglobin Electrophoresis

Principle of Alkaline electrophoresis [10]

Electrophoresis is the movement of charged particles in an electric field. The speed of movement depends on the electrical charge on each particle and the pH of the medium. At an alkaline pH of 8.4 to 8.6, haemoglobin is negatively charged and migrates toward the anode. Due to structural variations in their molecules, different haemoglobins possess different electrical charges and therefore separate during electrophoresis.

#### 2.5.2. Procedure (Method of Adu *et al.*, 2014 was adopted) [11]

A small quantity of venous blood was placed on a tile and mixed with two drops of water to lyse it. A small quantity of the haemolysate was placed on a cellulose acetate membrane and carefully introduced into the electrophoretic tank

containing Tris buffer. Electrophoretic separation was allowed to operate for 15-20 minutes at an electromotive force (e.m.f) of 300v. The results were read immediately against known haemoglobin (AC, SC and SS) which serves as controls.

**2.5.3. ABO and Rh Blood Grouping**

Tile method (Method of Ochei, 2007 was adopted) [10]

A drop of anti-A, anti-B, anti-AB and anti-D was placed on a clean white tile; a drop of blood from each subject was added respectively and mixed with each blood sample using glass rods. Blood groups were determined on the basis of agglutination of test serum by the respective antiserum.

Tube method (Method of Ochei, 2007 was adopted) [10]

One drop of washed 5% cell suspension was placed into four test tubes labelled A, B, AB and D, two drops of anti-A, anti-B, anti-AB, and anti-D were placed in the respective tubes. Each tube was mixed and incubated at room temperature for 10minutes. Blood groups were determined on the basis of agglutination by the respective antiserum.

**2.5.4. Rh D Negative Confirmation**

The Rh D negative blood was washed in normal saline three times, a drop was placed into a test tube, a drop of anti-D was added and incubated for 30minutes at 37°C, centrifuged and examined for agglutination, washed three times, two drops of AHG was added, centrifuged and re-suspended by tapping the tube and the result was interpreted based on presence or absence of agglutination was confirmed by adding IgG-sensitized cells [12].

**2.6. Statistical Analysis**

Graph pad instat computer statistical software was used for the analysis. Frequency distribution and percentage prevalence of all scores was generated. Chi-square and fisher exact test was performed. All statistical analyses were at 95% confidence interval at P≤0.05.

**3. Results**

A total number of 120 participants with different haemoglobin variants and forty (40) controls with normal haemoglobin (Hb AA) were involved in this study for the period of seven (7) months, the age of the participants range from 1-72 years. Fifty eight (58) among the participants were males and sixty two (62) were females.

From table 1, the pattern of ABO blood group indicated that; 18 (15%) of the participant were blood group A, 28 (23.33%) were blood group B, 16 (13.33%) were AB, and 58 (48.33) were O blood group. The male to female ratio for Blood

group A, B and AB were 1:1 each, while male to female ratio in blood group O among the participants were 13.5:15.5.

From Table 2 the pattern of Rhesus D blood group shows that; 114 (95%) of the participants were Rh D +, while 6 (5%) of them were Rh D -. The male to female distribution indicated that 49.12% of males were Rh D+, 50.88% of females were Rh D+, 33.33% of males were Rh D- and 66.67% of females were Rh D-.

Table 3 shows that 53.33% of Hb SS phenotype were males while 46.67% were females, 46.67% of Hb SC phenotype were males while 53.33% were females and 40.00% of Hb AC phenotype were males and 60.00% were females. This indicated that there were more males with Hb SS than females. But Hb SC and Hb AC indicated more females than males.

Table 4 shows higher percentage of blood group O among Hb SS and SC. Thirty two 32 (53.33%) of 60 participants with Haemoglobin SS had O blood group, 17 (56.67%) of 30 participants with Haemoglobin SC had blood group O. But the distribution of Hb AC was found to be higher in blood group B in which twelve 12 (40%) of 30 participants with haemoglobin AC had blood group B. It indicates that 18.33% of Hb SS had blood group A, 16.67% had blood group B, 11.67% had blood group AB and 53.33% had O blood group.

It shows that 6.67% of Hb SC had A blood group, 20.00% had B blood group, 16.67% had AB blood group and 56.67% had O blood group. It can be seen from the table that 16.67% of Hb AC had blood group A, 40.00% had blood group B, 13.33% had blood group AB, and 30% had blood group O

The table also indicates that 96.67% of participants with Haemoglobin SS, 93.33% of participants with Haemoglobin SC and 93.33% of participants with Haemoglobin AC were Rh D+, while 3.33%, 6.67% and 6.67% of participants with Haemoglobin SS, SC and AC respectively were Rh D-. Statistically negative association was established between Hb SS, Hb SC and Hb AC and the ABO and Rhesus D antigen Phenotypes in this study.

**Table 1.** Distribution of ABO blood group among Sex.

Blood groups	Males (%)	Females (%)	TOTAL (%)
A	9(50)	9(50)	18 (15)
B	14(50)	14(50)	28 (23.33)
AB	8(50)	8(50)	16 (13.33)
O	27(46.55)	31(53.45)	58 (48.33)
TOTAL	58	62	120 (100)

**Table 2.** Distribution of Rhesus D blood group among Sex.

Blood groups	Males (%)	Females (%)	Total (%)
Rh D +	56(49.12)	58 (50.88)	114 (95)
Rh D -	2(33.33)	4(66.67)	6(5)
TOTAL	58	62	120

Rh D+ = Rhesus D Positive Rh D- = Rhesus D Negative

**Table 3.** Distribution of Haemoglobin variants among Sex.

HB Phenotype	MALES (%)	FEMALES (%)	TOTAL
SS	32 (53.33)	28 (46.67)	60
SC	14 (46.67)	16 (53.33)	30
AC	12 (40.00)	18 (60.00)	30
TOTAL	58	62	120

HB=HAEMOGLOBIN

**Table 4.** Association between ABO and Rhesus D blood groups with Haemoglobin SS,SC and AC.

BG	Hb SS & HbAA			Hb SC and Hb AA			Hb AC and Hb AA		
	T (%)	C (%)	p-value	T (%)	C (%)	p-value	T (%)	C (%)	p-value
A	11(18.33)	8(20.00)	0.42	2(6.67)	8(20.00)	0.12	5(16.67)	8(20.0)	0.48
B	10(16.67)	9(22.50)	0.32	6(20.00)	9(22.50)	0.40	12(40.0)	9(22.5)	0.09
AB	7(11.67)	6(15.00)	0.29	5(16.67)	6(15.00)	0.46	4(13.33)	6(15.00)	0.44
O	32(53.33)	17(42.25)	0.19	17(56.67)	17(42.25)	0.18	9 (30.00)	17(42.25)	0.21
RhD+	58(96.67)	37(92.5)	0.39	28(93.33)	37(92.5)	1.00	28(93.33)	37(92.5)	1.00
RhD-	2(3.33)	3(7.50)	0.39	2(6.67)	3(7.50)	1.00	2(6.67)	3(7.50)	1.00

Key:BG = Blood Groups, T = Test, C = Control, Hb = Haemoglobin, Rh D+ = Rhesus D Positive, Rh D- = Rhesus D Negative

## 4. Discussion

The ABO blood group distribution among hemoglobin variants (Hb SS, Hb SC and Hb AC) reveals that blood group O was more prevalent which carries 48.33% followed by blood group B with 23.33%, blood group A with 15.00% and then AB with 13.33%. It indicated that blood group O is the predominant and blood AB was the least. The findings from this study are in agreement with other studies in Nigeria which also reported group O as the most prevalent and group AB as the least. Kalu *et al.*, (2012) [13] reported highest prevalence in group O (57%) and AB the least (1.69%) in Kano, Eru *et al.*, (2014)[14] reported group O in 52.5% and AB in 2.2% of their participant in Bebue, Tela *et al.*, (2015) [15] also reported group O being predominant (53.77%) and AB the least (10.38%) at Jama'are. Bakare *et al.*, (2006) [16] noted group O prevalence as 50% and AB as 5.9%, while Oluwadare and Shonekan (2008) [17] reported group O in 53% and AB in 3.9% of their participants. Group O prevalence of 55% and AB of 2.7% was reported by Adeyemo and Soboyejo (2006) [18]. The high prevalence of group O individuals in nature is of great advantage because of their status as universal donors. It is important to note that there has been an increase in the level of occurrence of blood demanding health conditions in many parts of sub-Saharan Africa (Uneke *et al.*, 2005) [19].

The distribution of ABO in Hb SS in this study indicates highest prevalence in blood group O followed by A, B, and AB, which is line with many researches which follows normal distribution including those mentioned above. In Hb SC, blood group O predominates followed by B, A and AB, this is in line with findings of Akhigbe *et al.*, (2009) [3] in a study of haemoglobin variants and ABO blood groups at Ogbomoso, Nigeria. In Hb AC blood group B was found to

be the most prevalent followed by O, A and AB. This highest prevalence of blood group B found in Hb AC is in line with study conducted by Garba *et al.*, (2016) [20] in AKTH, Kano which also reported highest prevalence of blood group B in Hb AC. This might be due to some relations that might exist between these haemoglobin variants and these blood groups.

The frequency of the Rhesus blood grouping antigen in this study was found in 95.0% of the study population while the remaining 5% were Rhesus negative. This finding agrees with studies from other parts of Nigeria which reported positivity of 96.7% (Jeremiah, 2006) [21], 94% (Adeyemo and Soboye, 2006) [18], 97.7% (Bakare *et al.*, 2006) [16], 93.2% (Akhigbe *et al.*, 2009) [3] and 93% (Erhabor *et al.*, 2012) [5]. The percentage of females with Rh D- (66.67) is higher than that in males (33.33%) though not statistically significant.

In this study, high prevalence of blood group O was found in Hb SS and Hb SC with percentage of 53.33% and 56.67% respectively, but statistical analysis shows no significant relation between them. It was also found in this study that blood group B was more prevalent in Hb AC carrying the highest percentage (40.00%), hence statistical analysis shows no significant relation between them.

## 5. Conclusion

This study shows that blood group O is more prevalent in Hb SS and Hb SC and blood group B was more prevalent in Hb AC.

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