



Frequency of Haemoglobin Genotype Variants, ABO and Rh 'D' Antigen among Madonna Undergraduates of South East Origin, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. RE, EIO and AN designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. CCNV, SOO, AMI, CJO and EFC managed the analyses of the study and managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Haemoglobin genotype variants, ABO and Rh blood groups are known to vary from one population to another. Standard electrophoretic and haemagglutination techniques were employed in testing the blood samples. Of the 150 test subjects screened, HbAA in the male subjects were 58(48.0%) and 63(52.0%) in the female counterparts. The frequency of distribution of HbAA, HbAS, and HbSS in the subject were 121 (80.7%), 28 (18.7%) and 1 (0.6%). Also HbAS in the female was 16(57.1%)

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while in male subjects was 12(42.9%). Blood group A (41.7%), group B(21.7%), group AB(28.6%) and group O(57.1%) were results for male subjects as against Group A(58.3%), Group B(78.3%), Group AB (71.4%) and Group O(42.9%) respectively in the female subjects.90% were Rh 'D' positive while 10% were Rh 'D' negative. Knowledge of the distribution of ABO, Rh 'D' blood groups and haemoglobin genotype variants in any population is useful in health care planning, medical diagnosis and targeting the population that need counseling. If such information is well managed it can make a difference in the quality of decisions that individuals will make especially as it concerns marriage, blood transfusion and other medical demands.

Keywords: Haemoglobin genotype variant; ABO; Rh 'D' antigen.

1. INTRODUCTION

ABO and Rhesus blood groups are among the most important blood groups clinically [1]. Landsteiner first described the ABO blood group in 1900 and it served the beginning of blood banking and transfusion medicine [2]. With the ABO blood group individuals are divided into four major blood groups namely, A, B, AB and O, according to the presence of antigens and agglutinins. Group A blood has type A antigens, group B blood has type B antigens and group O blood has neither A nor B antigens. Also plasma from blood group A contains Anti-B antibodies which act against type B antigens, whereas plasma from type B blood contains Anti-A antibodies, which act against type A antigens. Type AB has neither type of antibody and type O blood has both A and B antibodies [1]. Olugbemi et al., [3] reported that the antigens of ABO-Rh blood group and antibodies of ABO blood groups are found on the surface of red blood cell and plasma respectively. The authors further reported that two antigens and two antibodies are predominantly responsible for the ABO types.

It is a well-known fact that the ABO blood groups are not found in equal numbers [2]. In Caucasians in the United States, the distribution is group O, 47%, group A, 41%, group B, 9%, and AB, 3%. Among the African Americans the distribution is group O, 46%, group A, 27%, group B, 20% and group AB, 7%. In the Orientals the distribution is group O, 36%, group A, 28%, group B, 23%, and group AB, 13% [4]. In Ogbomosho, Oyo State Nigeria, 50% of the population are blood group O, 22.9% blood group A, 21.3% group B, and 5.9% group AB [5].

One of the antigens on the surface of red blood cells, the Rhesus antigen (named because a related antigen was first discovered in Rhesus monkeys), is found on the red cells of approximately 85% of the people of United States [1]. RhD antigen distribution varies from

one population to the other. RhD negative blood group is documented as 5.5% in South India, 5% in Nairobi, 4.8% in Nigeria, 7.3% in Lahore, 7.7% in Rawalpindi [1]. About 95% of African Americans are RhD positive. The haemoglobin contained in a quantity of blood accurately reflects the functional competence of the blood to supply oxygen to the tissue [6]. The structural abnormality may cause premature red blood cell destruction, easily denatured haemoglobin, haemoglobin with abnormal oxygen affinity, altered solubility and in some instances reduced globin synthesis.

Beside blood groups, haemoglobin genotype is also inherited blood characters [1]. Typically disorder associated with haemoglobin usually occurs within a set of population and it tends to vary according to locality. Makani et al., [7] reported that about 700 structural hemoglobin variants are known. Of these only 2 (Hb S, Hb C) has high frequencies in African continent. As such the major haemoglobin variants are AA, AS, AC, SS and SC [8]. Jeremiah [9] also reported that sickling disorders viz: heterozygous state for haemoglobin S commonly known as sickle cell trait, the homozygous state for HbS commonly referred to as sickle cell anaemia and compound heterozygous state for HbS in addition to haemoglobin C, D, E or other structural variants are found within the human population. For instances with the human population, Sickle haemoglobin (HbS) results from a substitution of one amino acid (Valine) for another amino acid (Glutamic acid) at position six of the β -globin polypeptide chain. This substitution is caused by a single-base mutation in codon 6 within the β -globin gene on chromosome 11, where the sequence GAG occurs instead of GTG [7]. Red blood cells contain glycoproteins and glycolipids that make up the blood group antigens on their surface [3] and Apecu et al., [1] opined that human blood groups depend on the functioning of glycosyltransferases (an enzymes that catalyze the formation of glycosidic bond

between monosaccharides). Several studies have been carried out to assess the frequency of ABO and Rhesus blood across ethnic groups, and population and results have shown diverging phenotypic characteristics [6]. The assessment of blood group ABO, Rhesus and genotype (AA, AS, SS) provide vital information during blood transfusion services. This will be vital in preventing hemolytic transfusion reactions and death, hemolytic disease of the fetus and newborn as well as to make for easy accessibility to rhesus negative blood for transfusion especially in cases of emergency.

1.1 Aim

The aim of this study was to evaluate the frequency of Haemoglobin genotype variants and ABO blood types among Madonna undergraduate of south east origin.

2. MATERIALS AND METHODS

2.1 Study Design

This research is a cross sectional study designed to determine the frequency of haemoglobin genotype variants, ABO and Rhesus blood types among Madonna undergraduates of south East origin.

2.2 Study Area

The study was carried out at Madonna University among undergraduates of south east origin, Nigeria.

2.3 Study Population

The study population was a total of 150 undergraduates of Madonna University comprising of 80 females and 70 males who were randomly recruited for the study.

2.4 Inclusion Criteria

Subjects that were considered eligible for this study are undergraduates of Madonna University.

2.5 Exclusion Criteria

Individuals who are not indigenes of the south east region in Madonna university were excluded from the study.

2.6 Sample Collection

Two milliliters of blood was collected from a prominent vein in the cubital fossa of the arm of subjects who participated in this study. A total of 150 blood samples were collected using a disposable plastic syringe using a standard venepuncture technique as described by Chesbrough (2008) into a commercial prepared concentration of Ethylene diamine tetra acetic acid (EDTA) container for the determination of blood group, rhesus group and haemoglobin variants. Each sample was mixed gently and thoroughly with the EDTA to prevent lyses and to ensure anticoagulation. The samples were analyzed within 2 hours of collection.

2.7 Methodology

2.7.1 Determination of Hb variant

2.7.1.1 Method

Alkaline cellulose acetate electrophoresis at pH 8.4-8.6 as described in the Helena Biosciences procedure manual.

2.7.1.2 Procedure

1. The cellulose acetate membrane was prepared as described in the Helena BioSciences procedure manual. About 100 mls of Tris EDTA borate buffer was placed equal amounts in each of the outer buffer compartment.
2. Two wicks were dipped in the buffer and drape one over each support bridge, ensuring contact is made by each wick with the buffer. The chamber was covered to prevent evaporation.
3. The cellulose acetate membrane was presoaked for at least 5 mins in the buffer and excess buffer removed by keeping the plate between blotting papers.
4. 5µl of each haemolysate sample (tests and controls) was transferred into the Zip-Zone well plate.
5. A cellulose acetate membrane (plate) was placed in the Zip-Zone aligning plate and the samples applied using the 8 unit applicator as described in the Helena BioSciences procedure.
6. The cellulose acetate membrane (plate) was immediately placed in the electrophoresis chamber.
7. The chamber was connected to the power

supply and electrophoresed for 25 minutes (or shorter) at 350V and 50mA

2.7.2 Determination of ABO antigen type

2.7.2.1 Method

Tile agglutination method using potent monoclonal anti-A, anti-B, and anti-D reagents (Plasmatec laboratories Ltd., Bridport, UK)

2.7.2.2 Procedure

A white tile was marked and divided as follows:

1. Anti-A, Anti-B and Anti-AB.
2. Into each division, 1 volume of anti-A, anti-B and anti-AB sera was pipetted.
3. To each division, one volume of 20% patient red cell suspension was added.
4. The contents of each division were mixed using a small clean piece of applicator stick for each. The tile was gently tilted from side to side and observed for agglutination.

2.7.3 Determination of Rh 'D' antigen

2.7.3.1 Method

1. Place a drop of antiserum (anti D antiserum) on a labeled white tile.
2. Add equal volume of the blood sample (20% cell suspension).
3. Mix well and rock gently for a maximum of two minutes.
4. Examine macroscopically and microscopically for agglutination.
5. Test a negative and a positive control in the same way.
6. Result: Agglutination- Rhesus D positive
7. No agglutination- Rhesus D negative

2.8 Statistical Analysis

These frequencies of the different parameter were determined using SPSS version 20. The data were expressed as percentage of the various categories.

3. RESULTS

A total of 150 participants were screened to determine the frequency of hemoglobin variants, ABO, and Rh 'D' blood groups. Out of total 150 subjects, male were 70(46.6%) and female 80(53.3%).

Table 1a and 1b revealed the distribution of the ABO blood groups among study subjects. Blood group O was found to be the most frequent 84(56%) while blood group AB was least frequent 7(4.7%). The frequencies pattern with respect to ABO can be shown as O > A > B > AB. There was a statistically significant relationship ($p>0.05$) in the distribution of ABO blood types and the gender tested.

Table 2a and 2b revealed the distribution of the Rhesus (D) blood groups among study subjects. In Rh blood typing, 135(90%) was Rh positive and 15(10%) was Rh negative. On further analysis male showed a relatively higher incidence of Rh negativity 10(66.7%) as compared to female 5(33.3%). There was statistically no significant difference ($p>0.05$) in the distribution of rhesus blood groups with the gender of the participants of this study

Table 3a and 3b showed different patterns of haemoglobin genotype variants according to gender. Out of 150 subjects, we found 121 subjects (80.67%) were normal (HbAA), 28 subjects (18.67%) were HbAS and 1 subject (0.67%) with abnormal hemoglobin variants or haemoglobinopathies. In present study, total observed haemoglobinopathy 35(23.3%), in which the most frequent haemoglobinopathy was HbAS 28 (18.6%) while less frequent Hb AC 3(2.0%). However, frequency of other hemoglobin genotype variants such as HbSS was 4(2.7%). In males, the frequency of abnormal haemoglobin HbAS was higher 12(34.3%) followed by HbAC 2(5.7%), followed by HbSS 1(2.9%). In females, the frequency of abnormal haemoglobin HbAS was higher 16(45.7%) followed by HbSS 3(8.6%), followed by HbAC 1(2.9%). There was statistically no significant difference ($p>0.05$) in the distribution of haemoglobin variants with the gender of the participants of this study.

4. DISCUSSION

Knowledge in hemoglobin variants and carrier status is thus necessary in reproductive decision making to modify risk for offspring of serious conditions such as sickle cell anemia as well as provide direct health benefit to carriers. The frequency of ABO Blood Groups is an important tool to determine the direction of recruitment of voluntary donors as required for each zone across the country.

In this study the ABO blood groups and Rh positivity in male and female showed that the Rhesus positive individuals were more prevalent than Rhesus negative individuals. The distribution of ABO blood group varies regionally, ethnically and from one population to another. In this study as in other studies it has been observed that 'O' is the most frequent (56%) and 'AB' as the least common (4.7%) blood group.

The second most common blood group was observed as "A" (24%) followed by "B" (15.3%) which is found to be consistent with the studies reported earlier [6]). For instance, Ugwu, [6] reported occurrence of blood group O, A, B and AB as 47%, 41%, 9% and 3% respectively in Caucasians in the United states, 46%, 27%, 20% and 7% respectively among the African Americans.

Table 1a. Frequency distribution of Blood Group among the subject

Blood groups	Frequency
A	36 (24%)
B	23 (15.3%)
AB	7 (4.6%)
O	84 (56%)
Total	150

Table 1b. Frequency distribution of Blood Group among Gender

Blood_group * gender cross tabulation					
			Gender		Total
			Female	Male	
Blood_group	A	Count	21	15	36
		% within Blood_group	58.3%	41.7%	100.0%
	AB	Count	5	2	7
		% within Blood_group	71.4%	28.6%	100.0%
	B	Count	18	5	23
		% within Blood_group	78.3%	21.7%	100.0%
	O	Count	36	48	84
		% within Blood_Group	42.9%	57.1%	100.0%
Total	Count		80	70	150
	% within Blood_Group		53.3%	46.7%	100.0%

Chi-Square Tests			
	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	10.729 ^a	3	.013
Likelihood Ratio	11.186	3	.011
N of Valid Cases	150		

a. 2 cells (25.0%) have expected count less than 5. The minimum expected count is 3.27.

P<0.05....significant

Table 2a. Frequency Distribution of Rh 'D' group among the subjects

Rhesus groups	Frequency
Positive	135 (90%)
Negative	15 (10%)
Total	150

Table 2b. Frequency Distribution of Rh 'D' groups among gender

Rhesus * gender cross tabulation					
			Gender		Total
			Female	Male	
Rhesus	negative	Count	5	10	15
		% within Rhesus	33.3%	66.7%	100.0%
	positive	Count	75	60	135
		% within Rhesus	55.6%	44.4%	100.0%
Total		Count	80	70	150
		% within Rhesus	53.3%	46.7%	100.0%

Chi-Square Tests					
	Value	Df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	2.679 ^a	1	.102		
Continuity Correction ^b	1.860	1	.173		
Likelihood Ratio	2.702	1	.100		
Fisher's Exact Test				.112	.086
N of Valid Cases	150				

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 7.00; b. Computed only for a 2x2 table ;p>0.05... (Not significant)

Table 3a. Frequency Distribution of Hb variants among the subjects

Hb Genotype	Frequency
AA	121 (80.7%)
AS	28 (18.7%)
SS	1 (0.6%)
Total	150

Table 3b. Frequency Distribution of Hb variants among gender

Gender * Genotype cross tabulation					
			Genotype		Total
			Female	Male	
Gender	AA	Count	63	58	121
		% within Gender	52.1%	47.9%	100.0%
	AS	Count	16	12	28
		% within Gender	57.1%	42.9%	100.0%
	SS	Count	1	0	1
		% within Gender	100.0%	0.0%	100.0%
Total		Count	80	70	150
		% within Gender	53.3%	46.7%	100.0%

Chi-Square Tests			
	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1.116 ^a	2	.572
Likelihood Ratio	1.499	2	.473
N of Valid Cases	150		

a. 2 cells (33.3%) have expected count less than 5. The minimum expected count is .47;p>0.05.... Not significant

Sometimes, slight variation occurs between gender for blood group A and B. For instance, Olugbemi et al. [3] reported blood group of 22.5 % (O), 7.5 % (A), 6.5 % (B) and 2 % (AB) among males, and 29 % (O), 11.5 % (A), 17.5 % (B),

and 3.5 % (AB) in females among adults attended Federal Medical Centre, Lokoja, Kogi State. Onuoha et al., [8] reported frequency of blood group A, B, AB and O as 20.3%, 22.7%, 3% and 54% in Yenagoa and its environs in

Bayelsa state. Jeremiah [9] reported frequency of blood group A, B, AB and O as 22.9%, 17.1%, 4.84% and 51.16% among students population of African descents in Port Harcourt, Nigeria.

The Rhesus negative and positive was 5(6.25%) and 75(93.75%) respectively for females and 10(14.3%) and 60(85.7%) for males. Typically, the frequency of Rhesus (D) negative is typically lower compare to the positive group. This trend has been reported in a rural southwestern Ugandan, Apecu et al., [1]. In Nigeria, Ugwu, [6] reported Rhesus positive (95.8%) and Rhesus

negative (4.2%) among Ebonyi state University students, Nigeria. Onuoha et al., [8] reported frequency of Rhesus positive (95.5%) and Rhesus negative (4.5%) in Yenagoa and its environs in Bayelsa state. Jeremiah, [9] reported frequency of Rhesus positive (96.77%) and Rhesus negative (3.23%) among students population of African descents in Port Harcourt, Nigeria. AB and Rh-negative had the least frequency. Olugbemi et al., [3] also reported that gender do not significantly affect both blood group system.

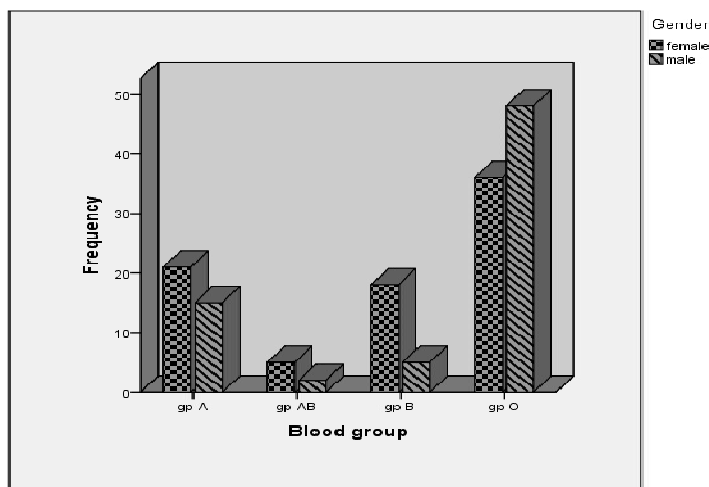


Fig. 1. Frequency Distribution of blood group among the gender

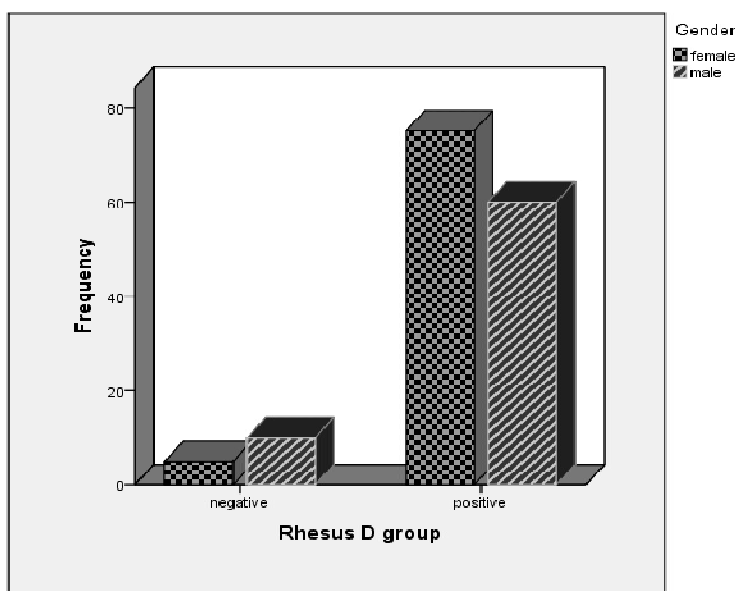


Fig. 2. Frequency Distribution of Rh'D' group among the gender

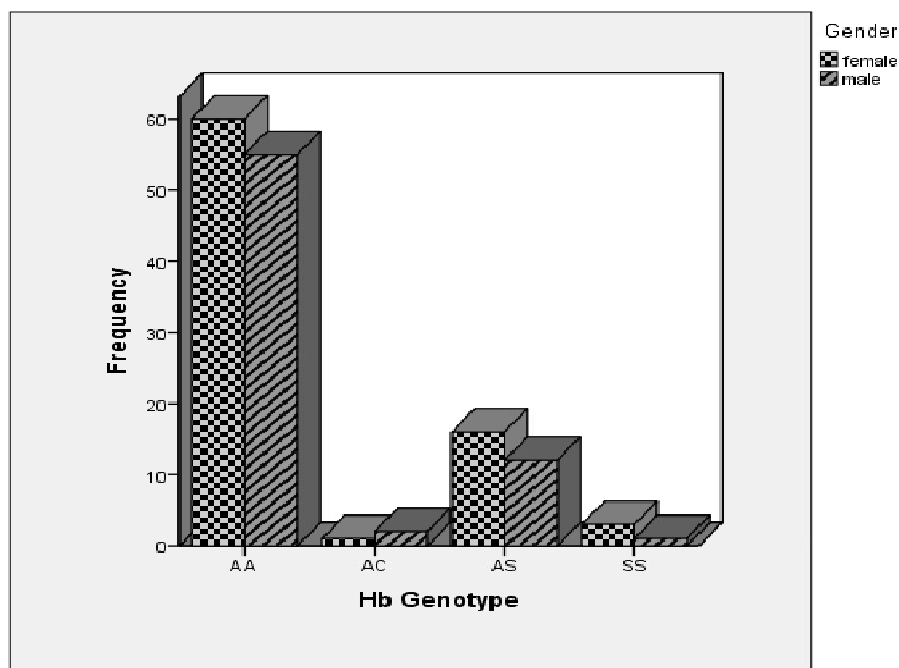


Fig. 3. Frequency Distribution of Hb variants among the gender

Olugbemi et al., [3] reported that when blood types are 100% genetically inherited, the environment can potentially determine which blood types in a population will be passed on more frequently to the next generation, which is done through natural selection.

Both males and females showed similar trend. Among the various haemoglobin variants (AA, AS, AC and SS) under study, the percentage occurrence were 60(75%), 16(20%), 1(1.3%) and 3(3.8%) respectively in females and 55(79%), 12(17.1%), 2(2.9%) and 1(1.4%) respectively in males. The trend of the haemoglobin variants in this study is comparable to the values previously reported in different locations in Nigeria. Jeremiah, [9] reported AA and AS haemoglobin variants as 80.32% and 19.68% among students of African descent in Port Harcourt. Onuoha et al. [8] reported frequency of AA, AS SS as 73.32%, 25.03 and 1.3% in Yenagoa and its environs, Bayelsa state. The lower values of SS among the population suggest that the SS gene is gradually reducing which could be due to enlightenment/genetic counseling prior to marriage [2]. Therefore, the knowledge of the various blood groups, rhesus D and genotypes is essential in diagnosis, genetic information, genetic counseling and general wellbeing of individuals Onuoha et al. [8,10-12].

5. CONCLUSION

Haemoglobin genotype variants, blood group and Rhesus system is the most vital human blood group systems necessary for blood transfusion. This study found the highest prevalence rate among the A, B, AB and O was blood group O in both sex under consideration. The Rhesus D typing also showed similar trend where Rhesus positive is more prevalent than Rhesus negative. Haemoglobin AA variants accounts for 52.2% in females and 47.8% in males. The results generally showed that individuals with blood group O, Rhesus positive and AA genotype were more among the target population. The result of this study will be useful health care planning and management processes as well as during blood transfusion services.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of

knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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