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STATISTICAL ANALYSES OF THE RHESUS D BLOOD GROUP

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ABSTRACT

Rhesus D factor is of serious clinical significance if a pregnant woman is negative, and her partner is positive, because it may result in Haemolytic Disease of the Newborn (HDN), Hyperbilirubinaemia or Erythroblastosis faetalis, due to Mother-Foetus Rhesus incompatibility. Of the 22,414 individuals sampled, 92.50 percent are Rhesus positive and 7.50 percent are Rhesus negative. In the entire sample, almost 53 percent of the individuals are estimated to be homozygous Rhesus positive (DD), while close to 40 percent are heterozygous (Dd), and 7.5 percent are Rhesus negative (dd). The highest frequency of the D allele was recorded in the North East, while the highest frequency of the d allele was recorded in the South South geopolitical zones.

Keywords: Hyperbilirubinaemia, HDN, Rhesus blood group

INTRODUCTION

The classical work of Landsteiner in 1901 (Landsteiner, 1961) which was not recognised until 1922, and which earned him a belated Nobel Prize for Medicine in 1930, is the bedrock of the different blood types we now have today. There are more than 25 different human blood group systems, however, the two commonest and most medically significant blood groups are the ABO and the Rhesus Blood Groups

This blood group system was so named, because it was originally thought to be similar to an antibody produced in rabbits when immunised with the rhesus monkey cells.

The Rhesus blood type was discovered about 40 years after the ABO blood group system, (Landsteiner and Wiener, 1940). They observed that during immunisation of Rabbits and Guinea pigs with blood from Rhesus Monkey and subsequent introduction of the antibodies produced by these animals to blood of humans, about 85 percent of the humans tested had their blood agglutinated by these antibodies and are thus classified as being Rhesus positive, while those whose blood were not agglutinated by the antibody were classified as Rhesus negative.

This blood group may be the most complex of all blood type systems, since it involves 45 different antigens on the surface of the red blood cells. Sir Ronald Fisher in 1944 proposed the current system of naming for this blood group and labelled the three sets of alleles as c/C, d/D and e/E (Giangrande, 2000). One haplotype consists of only one allele from a set, thus it is only individuals whose genotype is cde/cde that are classified as Rhesus negative.

Despite its actual genetic complexity, the inheritance of this trait is straightforward and predictable, and the model is thought of as a locus with two alleles D and d, yielding individuals who may be homozygous dominant (DD) or heterozygous (Dd); both are Rhesus positive and those who are homozygous recessive (dd); these are negative (i.e. they do not have the D antigen). In this respect, it is now a common phenomenon to only refer to the D gene and thus use of the term Rhesus D. This is due to the fact that the D gene is the most clinically significant of the three alleles and the lack of it in an individual, stimulate such individual into producing harmful antibodies.

Unlike the ABO system, where Mother-Foetus incompatibility problems are very rare (less than 0.1 percent of births are affected) and its symptoms not severe when they do occur, extreme care has to be taken in the case of Mother-Foetus Rhesus incompatibility.

Rhesus disease / Haemolytic Disease of the Newborn (HDN) / Hyperbilirubinaemia or Erythroblastosis faetalis are consequences of the Mother-Foetus incompatibility. This disease arises as a result of a Rhesus negative mother carrying a Rhesus positive foetus in pregnancy. In a typical Mendelian segregation, it is possible for an heterozygous Rhesus positive father to either contribute a d or D gene, thus if the D gene is produced and the mother is Rhesus negative, it means that the unborn foetus will be heterozygous Rhesus positive (Dd). The presence of the D antigen in the foetus, which may be leaked into the mother's blood via placental bleed (Faeto-maternal haemorrhage) or at child birth, will elicit production of antibodies by the mother's immune system because of the alien D antigen in her blood. This formation of antibodies (alloimmunisation) typically occurs after delivery of the baby at the end of pregnancy, however, elective abortion or spontaneous miscarriage can also result in antibody formation. This sensitisation of the mother's immune system may have dire consequences in subsequent pregnancies of the woman, especially when the Rhesus group of the foetus is positive.

Due to the significance of this disease, several methods of prenatal diagnosis and prevention have been outlined, also effective treatment methods are globally in use. Education and enlightenment have equally played a significant role in the control and reduction of these diseases.

Genetically, the Rhesus locus is composed of two homologous structural genes on chromosome 1 (Colin et al, 1991). The Rhesus group antigen are derived from two genes (RHD and RHCE) located at chromosomal position 1p34.1 – 1p36 i.e. the short arm, region 3, band 4, sub-band 1 through band 6 of chromosome 1 (Wagner and Flegel, 2000).

This study is aimed at the following:

- Examine the prevalence of the rhesus negative amongst the various ethnic groups within Nigeria and
- Establish if the observed values conform to expectations in a randomly mating population and test their deviations from theoretically estimated values based on the Hardy Weinberg Equilibrium.

MATERIALS AND METHODS

The source of the data, and the statistical packages used in this analysis is as described in Abanikannda *et al*, (2004).

Phenotypically, the rhesus D factor could either be present or absent (positive or negative) in individuals, however, along with the recessive allele Rh D negative (dd), there are other possible forms for the Rh D positive. This could be the homozygous DD, or heterozygous Dd or dD.

The dominance of the D allele over the d allele makes phenotypic classification of the Rhesus D positive impossible. However, the EM algorithm technique could be used to determine the proportion of heterozygote in each of the ethnic regions and the population assuming a Hardy - Weinberg equilibrium.

The frequency of the recessive homozygous allele (dd) is represented with q and the positive Rh D (DD, dD or Dd) is represented by p where $p + q = 1$.

RESULTS AND DISCUSSION

The observed frequencies for the rhesus D factor are presented in Table 1. The proportion of rhesus negative individuals varies across the regions from 6.4 percent in the North East to 10.8 percent in the South South. The average proportion of rhesus negative is 7.5 percent in this study.

Table 1: Frequency distribution of Rhesus D Factor in Nigeria

Ethno-Geographical regions	Observed Phenotypic Frequencies		
	Rh D+ (Rhesus Positive)	Rh D- (Rhesus Negative)	TOTAL
South West	14826	1162	15988
South East	4451	361	4812
South South	867	105	972
North Central	188	14	202
North East	44	3	47
North West	356	37	393
Total	20732	1682	22414

The EM Algorithm was used to estimate the proportion of the rhesus positive individuals that is homozygous or heterozygous (Table 2).

Table 2: Expected Genotypic Frequencies of the Rhesus D Factor

Ethno-Geographical regions	Expected Genotypic Frequencies		
	DD (Positive)	Dd (Positive)	dd (Negative)
South West	0.5335	0.3938	0.0727
South East	0.5272	0.3978	0.0750
South South	0.4507	0.4413	0.1080
North Central	0.5428	0.3879	0.0693
North East	0.5585	0.3776	0.0638
North West	0.4805	0.4254	0.0941
Total	0.5272	0.3978	0.0750

In the entire population sampled, almost 53 percent of the individuals are homozygous rhesus positive (DD), while close to 40 percent are heterozygous (Dd), 7.5 percent of the individuals are rhesus D negative (dd). The highest frequency of the D allele was recorded in the North East, while the highest frequency of the d allele was recorded in the South South.

Since the clinical implication of the rhesus D negative factor is sex limiting, further analysis of the distribution of the rhesus D factor by sex of individuals sampled becomes imperative.

Out of the 22,414 records that had information on Rhesus factor, only 21,813 had additional information on the sex of the individuals sampled (Table 3).

Expectedly the sex ratio is more favourable to the females, since the original data contains more of females than male. The frequency of occurrence of the rhesus D negative within the sexes revealed that the proportion ranges from as low as 4.9 percent rhesus negative females in the North Central to as high as 14.0 percent of rhesus D negative male in the South South.

Table 3: Frequency distribution of Rhesus Factor by Region and Sex

Ethno-Geographical regions	Observed Phenotypic Frequencies		
	Rh D+ (Rhesus Positive)	Rh D- (Rhesus Negative)	TOTAL
South West	14457	1130	15578
Male	3266	280	3546
Female	11191	850	12041
South East	4304	345	4649
Male	1094	79	1173
Female	3210	266	3476
South South	850	102	952
Male	221	36	257
Female	629	66	695
North Central	175	13	188
Male	39	6	45
Female	136	7	143
North East	44	3	47
Male	11	0	11
Female	33	3	36
North West	355	35	390
Male	95	13	108
Female	260	22	282
Total	20185	1628	21813
Male	4726	414	5140
Female	15459	1214	16673

A 2 x 2 contingency analysis for independence of rhesus factor on sex for each of the regions revealed that, with the exception of the South South, which was significant ($P<0.05$), the chi square for the other regions were not statistically significant ($P>0.05$). This led to another test of independence between the rhesus factors and the ethno-geographical zones (Table 4).

Over 80 percent of the total deviation from expectation obtained in this study is due to the very large differences between the observed and expected values for the rhesus factors in the South South region. This large value from the South South contributed greatly to the overall chi square obtained in this analysis, thereby making the deviation significant ($P<0.05$). If however, the South South is not included and the North East removed from the analysis because of its expected frequency less than 3, the distribution of Rhesus factor in the study is not dependent on the regions where the individuals came from. The unusually high d allelic frequency for the South South may be responsible for this observation.

Table 4: Test of Independence between Rhesus factors and Regions.

Ethno-Geographical regions	Rhesus Positive		Rhesus Negative		$\frac{(o-e)^2}{e}$
	Observed	Expected	Observed	Expected	
South West	14826	14788.22	1162	1199.78	1.2860
South East	4451	4450.90	361	361.10	0.0000
South South	867	899.06	105	72.94	15.2335
North Central	188	186.84	14	15.16	0.0957
North East	44	43.47	3	3.53	0.0851
North West	356	363.51	37	29.49	2.0667
Total	20732	20732.00	1682	1682.00	$\sum \frac{(o-e)^2}{e}$ =18.7671