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ORIGINAL ARTICLE

Haemoglobin Haplotypes and Probable Allelic Frequencies of natives of Okolobiri Community, Nigeria: Population genetics study

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Abstract

Introduction: Sickle cell disease is an autosomal recessive disorder in which patients inherit one mutated copy of the β-globin gene from each parent. This inheritance results to sickle-shaped red blood cell and associated fragility which results in the destruction of the patients red blood cells. This project was carried out as a population genetic study to determine the frequencies of the haemoglobin haplotypes (A and S) and allelic frequencies with a view to predicting the dynamics of the abnormal genes using a typical African community as a case study.

Materials and Methods: Two hundred and twenty-eight (228) subjects constituted the study population of which one hundred and sixty four (164) were males (28%). Haemoglobin electrophoresis membrane at a pH 8.6 was used to establish the haemoglobin electrophoretic pattern of the study population.

Results: The haemoglobin electrophoretic pattern of the study participants were as follows: Hb AA (60.5%), Hb AS (25.5%) and HbSS (14%). The distribution among males were; HbAA (62.2%), HbAS (29.3%) and HbSS (8.5%). and females: Hb AA (56.3%), HbAS (15 .6%), HbSS (28.1%). The HbA haplotype frequency was 0.732 while the HbS was 0.268. the allelic frequencies obtained by Hardy-Weinberg calculation were as follows. HbAA (0.536), HbAS (0.392) and Hb SS (0.072). The χ^2 value of 29.475 obtained in this study was higher than the 3.84 at 5% significant level, hence, the null hypothesis that the population is not in Hardy-Weinberg equilibrium was accepted..

Conclusion: The study revealed high homozygous HbSS inheritance among females (28.1%) meaning that females are more affected than the males. The frequency of HbAS is expected to increase as this population is not in Hardy-Weinberg equilibrium. More awareness and sensitization is needed in our communities in order to reduce the prevalence of sickle cell anaemia in our African society.

Keywords; haplotypes, allelic frequencies, population genetics, Okolobiri, Nigeria.

Introduction

Population genetics is the study of **variation within populations of individuals**, and the forces which shape it. This involves studying changes in the frequencies of genetic variation in populations over space and time(1). One of the most important principles of population genetics is Hardy-Weinberg Equilibrium (HWE) which states that "genotype frequencies in a population remains constant between generations in the absence of external or outside factors" (1). Going by the definition of HWE, it means that for a locus with two alleles A and a with corresponding frequencies of p and q, three genotypes are possible which are AA, Aa and aa and the expected frequencies will be p^2, 2pq and q^2 respectively (2). Deviations from HWE can be caused by a lot of factors including mutation, natural selection, non-random mating, genetic drift and gene flow (2). Heterozygote deficiency or excess can also occur as a result of positive or negative assertive mating resulting to deviation from HWE (3). Non-random mating due to geographical location might also be a common cause of deviation from HWE due to hetrozygous deficiency in large populations of different ethnicities (4). If a population consists of several sub-populations and individuals randomly mate within but not between subpopulation, then homozygous alleles in the overall population will be observed more frequently than expected by HWE (5).

In African setting, the presence of a recessive disorder or disease causing variant at a high frequency in populations may also be due to over dorminant selection i.e a heterozygote variant provides some advantage to carriers (6). As in the case for HBBC.20A>T, which provides carriers protection from malaria (MIM.611162) (7).

The normal haemoglobin types of any given population consist of haemoglobin A (HbA) which contains two alpha (α) and two beta (β) globin chains; haemoglobin A_2 (HbA_2) which contains two alpha (α) and two delta (δ) protein chains and makes up about 2% - 3% of haemoglobin types in adults and haemoglobin F (HbF) which contains two alpha (α) and two gamma (ɣ) protein and makes up to 2% of haemoglobin found in adults. HbF is the primary haemoglobin produced by the foetus during pregnancy and its production usually falls to a low level shortly after birth.(8)

Haemoglobin variants can occur when genetic changes cause alterations in the amino acids that make up the β globin protein. These changes may affect the structure of the haemoglobin, its production rate and /or its stability (9).

The most common alpha-chain related condition is alpha thalassemia (8,9). The β chain haemoglobin variants are inherited in an autosomal recessive fashion. This means that the person must have two altered gene copies, one from each parent to have a haemoglobin variant related disease. One normal beta gene and one abnormal beta genes inherited makes the individual heterozygous for the abnormal haemoglobin and a carrier. Two abnormal beta genes of the same type when inherited makes the individual homozygous and consequently would produce the associated haemoglobin variants with symptoms of diseases(10). Two abnormal beta genes of different types when inherited makes the individual doubly or compound heterozygous. The affected patients manifest symptoms related to one or both of the haemoglobin variants that he/she produces (9,10).

Several hundred beta chain haemoglobin variants have been documented. Few common and clinically significant ones are as follows:

Haemoglobin S-This is the primary haemoglobin in people with sickle cell disease. The presence of haemoglobin S causes the red blood cell to deform and assume a sickle shape when exposed to decreased amounts

of oxygen. These sickle cells can occlude the blood vessels causing pain and impaired circulation, decreased oxygen carrying capacity and decrease the cells life span. (8,9,10)

Haemoglobin C – Present in about 2-3% of West African descent who are heterozygous for haemoglobin C. HbC disease caused minor amount of haemolytic anaemia and a mild to moderate enlargement of the spleen.

Haemoglobin E – one of the most common beta chain haemoglobin variant in the world and most prevalent in the South East Asia.

Less common haemoglobin variants include Haemoglobin D, Haemoglobin J, haemoglobin M. Additional examples of beta chain variant include Haemoblogin F which is the primary haemoglobin produced by the foetus and its primary role is to transport oxygen efficiently in a low oxygen environment. Production of haemoglobin F stops at birth and decreases to adult levels by 1 – 2 years of age. HbF may be elevated in several congenital disorders. Levels can be normal to increased in beta thalassemia and are frequently increased in individual with sickle cell anaemia and sickle cell – beta thalassemia. Haemoglobin F levels are also increased in a rare condition called hereditary persistence of foetal haemoglobin (HPFH).

Haemoglobin H – An abnormal haemoglobin that occurs in some cases of alpha thalassemia. It is composed of four beta (β) globin chains and is produced in response to severe shortage of alpha (α) chains. Haemoglobin Barts – Develops in fetuses with alpha thalassemia. Formed from four gamma (ɣ) chain hen there is a shortage of alpha chains. An individual can also inherit two different abnormal genes, one from each parent. Examples are Haemoblogin SC diseases, sickle cell – Haemoglobin D disease, Haemoglobin E – Beta thalassemia, Haemoglobin S – Beta thalassemia etc.(9,10,11,12)

Godfrey Hardy and Wilhelm Weinberg developed a simple equation that can be used to discover the probable allelic frequencies in a population and to track their changes from one generation to another (1,2). In this equation ($\rho^2 + 2\rho q + q^2 = 1$), ρ is defined as the frequency of the dominant allele and q as the frequency of the recessive allele for a trait controlled by a pair of alleles (A and a). In other words, p equals all of the allele in individuals who are homozygous co-dominant (AA) and half of the alleles in people who are heterozygous (Aa) for this trait in a population. In mathematical terms, this is ρ = AA + ½ Aa

Likewise, q equals all of the alleles in individual who are homozygous recessive (aa) and the other half of the alleles in people who are heterozygous (Aa). q = aa + ½ Aa.

Because there are only two alleles in this case, the other must equal 100% = ρ + q = 1

Since this is logically true, then the following must also be correct.ρ = 1 – q

There were only a few short steps from this knowledge for Hardy and Weinberg to realize that the chances of all possible combination of alleles occurring randomly is $(\rho + q)^2 = 1$

Or more simply $\rho^2 + 2\rho q + q^2 = 1$

In this equation

ρ^2 = predicted frequency of homozygous dominant (AA) people in a population

$2\rho q$ = predicted frequency of heterozygous (Aa) people and ρ^2 = predicted frequency of homozygous recessive (aa) ones.

Haemoglobinopathies are the most commonly genetically inherited disorders in Africa (19). WHO estimates that approximately 5% of world's population are being carriers and more than 12,000 infants born with a major and clinically significant haemoglobinoapthy(19). Several studies have reported the frequencies of haemoglobin

variants, but that of this homogenous rural community has not been reported. This study was aimed at determining the current status of the frequencies of abnormal haemoglobin variants and predict the gene frequencies in the population using the Hardy-Weinberg mathematical equation.

Materials and Methods

Study Area: This study was conducted at Okolobiri Town in Gbarain Clan in Bayelsa State, Nigeria. The community is located in the geographical coordinates of latitude 5.0481 (5″ 2′ 53″N) and longitude 6.32631 (6″ 19′ 34″E).

Study Population: The study population comprised of two hundred and twenty eight (228) adults One hundred and sixty four (164) were males while the remaining 64 were females. They were all recruited into the study while attending the Niger Delta University Teaching Hospital (NDUTH), a tertiary healthcare institution located within the Okolobiri community.

Ethical Approval: The study received an institutional ethical approval from the NDUTH ethical committee.

Informed Consent: All the participants in this study gave their informed consents before blood samples were collected from them.

Haemoglobin Electrophoretic Pattern

Principle: The haemoglobins in the blood sample are separated by electrophoresis using an alkaline buffer (pH 8.6) on cellulose acetate membrane and stained with ponceaus.

Procedure: Two milliliters of blood was collected from the dorsal vein of the subjects and put into EDTA bottle and mixed gently. A portion of the blood sample was put in a clean khan tube and washed three times with normal saline (0.85% sodium chloride). Six drops of haemolysate were added to the sediment and allowed to stand for 3 minutes so as to lyse the blood same. The lysed samples were applied on celluose acetate paper using an applicator and the paper was placed in the electrophoretic tank containing Tris-EDTA-Borate buffer at pH 6.8. The electrophoretic separation was allowed at room temperature for 30 minutes at 220v as the electromotive force. A commercially prepared known haemoglobin (Hb A, S and C) were run as controls along with the test and the results were read immediately after the end of the test time and results reported.

Calculation of allelic frequencies: The probable allelic frequencies were calculated mathematically using the Hardy-Weinberg equation

$$\rho^2 + 2\rho q + q^2 = 1.$$

Results

Two hundred and twenty-eight (228) individuals constituted the study population of this, 164 (72%) were males while 64(28%) were females. The distribution of haemoblogin electrophoretic pattern were obtained as follows AA 138(60.5%), AS 58(25.5%), SS 32(14%) (Table 1).

From the frequency distribution above, the allelic frequencies for the given locus (n = 228). Each individual from the study with AA has two copies of the A allele, therefore, the 138 individual with this pattern have a count of 376 A alleles. Heterozygote individuals (AS) have one of each allele, so there are 58A alleles and 58 S alleles among them.

Like the AA homozygotes, individuals with SS pattern have two copies of the S allele, so these 32 individuals contribute 645 alleles to our count. In other words, among the 228 individuals in this study, there are 334 A alleles and 122 S alleles.

To calculate the allelic frequencies, we simply divide the number of A or S alleles by the total number of alleles as follows:

334/456 = 0.732 = ρ = frequency A allele.

122/456 = 0.268 = q = frequency of S allele (Table 3)

If this population were in Hardy-Weinberg equilibrium, we would expect the genotype frequencies for AA, AS and SS to be ρ^2, 2ρ and q^2 respectively.

$\rho^2 = (0.732)^2 = 0.536$

$\rho q = 2(0.732)\ (0.268) = 0.392$

$q^2 = (0.268)^2 = 0.072$

for the 228 individuals in this study, then we would expect that approximately 122 individuals ρ^2 x n = 0.536 x 288 = 122.208 would have the AA pattern.

90 individuals (pq x n = 0.392 x 288 = 89.376) would have the AS phenotype and 16 individuals q^2 x n = 0.072 x 228 = 16.416) would have the SS genotype.

The expected values compared the observed values for the genotype frequencies at a given locus using the chi-square will be as

$$x^2 = \frac{\sum(0-E)^2}{E}$$

$$= \frac{(138-122)^2}{122} + \frac{(58-90)^2}{90} + \frac{(32-16)^2}{16}$$

$$= \frac{16^2}{122} + \frac{(-32)^2}{90} + \frac{(16)^2}{16}$$

$$= 2.098 + 11.377 + 16$$

$$\chi^2 = 29.475$$

There is 1 degree of freedom, the 5% significance level for 1 degree of freedom is 3.84 and since the χ^2 value is higher than this, the null hypotheses that the population is not in Hardy-Weinberg equilibrium stands and is accepted

Interpretation: Checking our mathematics to ensure the correct genotype frequencies have been calculated, $\rho^2 + 2pq + q^2$ should equal 1, therefore $(0.732)^2 + 2(0.732)\ (0.268) + (0.268)^2 = 1$.

Similarly, ρ + q must equal 1, therefore 0.732 + 0.268 = 1.

Discussion

This study was carried out with the aim of providing an update on the distribution of haemoglobin variants, haemoglobin haplotype frequencies and probable gene frequencies in an Ijaw community in Nigeria.

The percentage distribution of HbAA in this study was 60.5%. this value was lower than 80.32% reported by Jeremiah (13) some sixteen years ago. The implication being that the normal haemoglobin (HbAA) in our typical African setting is gradually declining. The percentage distribution of HbAS in this study was 25%. The value is higher than 22.9% obtained in Jeremiah's study sixteen years ago (13) and lower than 29.4% reported by Erhabor *et al* (14). Those with homozoygous Hbss in this study was 14%. This valued is very high compared to 1.5% reported in Erhabor *et al.*(14). Four years later, in 2006, the value increased to 1.5% and now in this study as high as 14% is being reported. All the

Table 1: Distribution of haemoglobin phenotypes among the study population

Hb Genotype	Number (%)
Hb AA	138 (60.5%)
Hb AS	58(25.5%)
Hb SS	32(14%)
Total	228(1000%)

Table 2: Distribution of haemoglobin phenotypes by gender among study participants

Hb Genotype	Gender		Total	χ^2
	Males	Females		
Hb AA	102(62.2%)	36(56.3%)	138(61%)	1.049
Hb AS	48(29.3%)	10(15.6%)	58(25%)	5.689*
Hb SS	14(8,5%)	18(28.1%)	32(14%)	8.00**
Total	164(100%)	64(100%)	228(100%)	

* = significant at p<0.01; ** = significant at p< 0.001

Table 3: Calculated Allelic frequencies in the study population

Alleles	**Number observed**	**Total Alleles**	**Allele Frequency**
Hb A	334	456	0.732
Hb S	122	456	0.268

Table 4: Probable Haemoglobin phenotype frequencies in the study population using Hardy-Weinberg equation

Genotypes	**Number**	**Gene frequencies**	χ^2	**p-value**
Hb AA	Observed (123) Expected (122)	0.536		
			29.475	0.0001
Hb AS	Observed (58) Expected (90)	0.392		
Hb SS	Observed (32) Expected (16)	0.072		

studies mentioned above were conducted in the same south-south region of Nigeria and predominantly among the Ijaws.

Contrastingly, the prevalence of a sickle cell trait in this study (25%) is still higher than 18.1% reported in the South-West region of Nigeria by Oyeyemi *et al.*(15). It means that the heterozygous HbAS prevalence is rapidly increasing in our population irrespective of the geographical regions in Nigeria.

Using the Hardy-Weinberg equation to predict the gene frequencies in this population, it was discovered that an increase of the HbAS gene is expected in this population while there will be a decline in the normal hemoglobin (HbAA) and sickle cell disease is a clinical syndrome caused by the presence of haemoglobin S (HbS) in which glutamic acid in position 6 of the β chain of haemoglobin is substituted by valine (β Glu b val). It is generally recognized as an autosomal recessive disorder, in that individuals who have inherited one copy of the HbS allele and one normal HbA allele (ie have HbAS or sickle cell trait) are typically asymptomatic and spared the serious complication associated with possessing two copies of the mutant allele (HbSS) (16) while HbAS represent an asymptomatic carrier state, clinical and epidemiological studies have shown that sickle cell trait is certainly not an entirely harmless condition. (17) The presence of HbS in sickle cell trait may contribute to specific disease processes particularly under extreme conditions that promote HbS polymerization.(17). HbAS can pose a diagnostic challenge but elucidating their molecular basis provides further insight into the pathophysiology of sickle cell disease and help identify genetic risk modifiers in sickle cell trait (17,18). One thing that stands out in this study is the heterozygous (HbAS) gene is on the increase in our typical African population while the normal HbAA gene declines.

Conclusion: The study revealed high heterozygous HbSS inheritance among females meaning that females are more affected than the males. The frequency of HbAS is expected to increase as this population is not in Hardy-Weinberg equilibrium. More awareness and sensitization is needed in our communities in order to reduce the prevalence of sickle cell anaemia in our African society.

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