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Prevalence of sickle cell disease/ABO blood group among secondary school students: A case study of government day secondary school (GDSS) mile six Jalingo, Taraba state, Nigeria

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Abstract
This study was carried out to determine the haemoglobin electrophoretic patterns and distribution of ABO blood group among randomly selected students attending Government day secondary school Howui mile six Jalingo, Taraba State Nigeria. One hundred and thirty-nine (139) students selected at random constituted the subjects for this study. The standard method of cellulose acetate electrophoresis was used to determine the haemoglobin. A small quantity of haemolyste of vein puncture blood from each of the subjects was placed on a cellulose acetate membrane and carefully introduced into the electrophoretic tank containing Tris-EDTA-borate buffer at pH 8.6.
For the Haemoglobin phenotype ABO, ADAM Standard tile method of antigen-antibody reaction with the use of Anti-sera was used to examine the presences of agglutination. The result showed that; (106) 76.26% have HbAA haemoglobin, (32) 23.02% have HbAS haemoglobin, (1) 0.72% have HbSS haemoglobin. ABO blood group screened were as follows: group A^+ (35) 25.18%, group B^+, (30) 21.58%, group AB^+ have (8) 5.76%, group O^+ have (55) 39.57% were Rh. 'D' positive while the rest are Rh 'D' negative 2.87% (4) were group B- 1.44% (2) were group AB^- 3.59% (5) were group 0^-.

Keywords: Haemoglobin, tris-EDTA, electrophoresis, Rhezus, HbAA, HbAS, HbSS, ABO

1. Introduction

Sickle cell disease (SCD) is an inherited blood disorder caused by abnormal hemoglobin [1]. Sickle cell disease limits the oxygenating role of hemoglobin, resulting in the damaging or the "sickling" of the red blood cells [2]. This disorder affects all parts of the human body and differs widely among individuals [3]. In 1910, Dr. James Herrick, a Chicago physician, was the first American to formally report and identify elongated, sickle-shaped hemoglobin in an' anemic Grenadian student's blood smear. Herrick coined the now familiar term "sickle cell" [4].

The sickle shaped red blood cells described by Herrick caused several complications, including chronic anemia, vaso occlusive pain episodes, ischernic organ damage, infections, small stature, and delayed puberty [2]. For many generations sickle cell disease has been a prevalent disorder in Africa. Reports show that sickle cell disease was a well-known disorder in West Africa and that the West African natives had several local names for this disease before it was discovered in America [5]. Sickle cell disease affects millions of people throughout the world, and it is found to be the most common blood disorder among families whose ancestors came from Sub-Saharan Africa, South America, Cuba, Central America, Saudi Arabia, India, and the Mediterranean regions [1]. Studies indicate that approximately 1 in 12 African-Americans are heterozygous for the disorder, and approximately 1 in 500, African-American newborns are diagnosed annually with SCD [6]. Also, the life expectancy for SCD has doubled since the 1960s. Before that time, few patients lived to reach adulthood [7]. It was not until the 1970s that this blood disorder began to capture public attention in the United States. Prior to that time, many researchers held numerous misconceptions about the nature and course of the disease. Richard Nixon was the first president to make sickle cell disease a matter of national concern by signing the Sickle Cell Anemia Control Act of 1972 [8].

In 1971, President Nixon focused his health message to Congress on sickle cell disease, which at that time was a virtually unknown inherited blood disorder in the African-American community [5]. The 1972 act set the foundation for funding toward sickle cell screenings, counseling programs, and the development and distribution of sickle cell anemia educational materials to the general public [9]. With the help of President Nixon, several sickle cell disease research organizations were created, such as the Sickle Cell Disease Association of America (SCDAA), which was established by Charles Whitten in 1972. The SCDAA was designed to improve the quality of life for patients and families with sickle cell disease [5]. After the 1970s, the public's focus unfortunately shifted once again. The new law, which first established sickle cell education, genetic screenings, and counseling, was stated to be "fraught with controversy". Even the African-American community, which has a higher probability of inheriting SCD, began to regard informed reproductive decision methods, such as screening and counseling, with trepidation and distrust [10]. After President Nixon turned sickle cell disease into a national priority, legislators quickly began to pass laws that mandated premarital and pre-school screenings for sickle cell disease. The U.S Air Force began to deny any men, who were diagnosed as carrying the sickle cell trait, occupational opportunities if they were applying to be pilots or co-pilots, and insurance companies even increased premiums for

individuals with the trait.

The American people began to view these new legislative policies as genocidal, and these policies were eventually overturned [5]. The lack of national concern for SCD created a barrier in health care. Complications due to the sickling of the red blood cells therefore continue to be a significant issue to patients and physicians in today's medical world. Physicians remain puzzled by the biological and clinical intricacies of SCD, and SCD researchers are trying to find a cure to reverse the "sickling effect" in the human body.

All human red blood cells contain a pigmented metalloprotein called haemoglobin which is of different types. Hemoglobin is the oxygen carrying pigment of the red blood cells. Defects in its genes produce abnormal haemoglobin which leads to conditions known as haemoglobinopathies. Haemoglobin electrophoretic patterns include the normal haemoglobin which is the most prevalent and is referred to as hemoglobin A (HbA) and other abnormal ones also exist, like hemoglobin S (HbS), which is a variant form of the normal haemoglobin. The variation is in the β-globin chain gene, causing a change in the properties of haemoglobin which results in sickling of red blood cells.

Another variant is haemoglobin C (HbC), which also occurs as a result of a variation in the β-globin chain gene. This variant presents with mild chronic hemolytic anemia (in homozygous HbCC and in double heterozygous SC). Sickling disorders include the heterozygous state for haemoglobin S or the sickle cell trait (AS), the homozygous state for HbS or sickle cell anemia (SS) and the compound heterozygous state for HbS together with other haemoglobin (C, D, E) or other structural variants [11]. The prevalence of sickle cell anaemia (HbSS) among the Black population in the United States is reported to be 9% [12] and 30% — 40% generally for Africans [13]. These hemoglobin variants cause moderate to severe haemolytic anemia leading to high degree of morbidity and mortality [14].

Sickle-cell disease (SCD) or sickle-cell anaemia (SCA) is an autosomal recessive genetic blood disorder characterized by red cells that assume an abnormal, rigid, sickle shape. Sickle cell disease causes polymerization of haemoglobin resulting in vaso-occlusive, a plastic, sequestration and haemolytic crisis. It is caused by a single point mutation in the β-g1obin chain of the haemoglobin molecule and result from a substitution of the hydrophilic amino acid glutamic acid by the hydrophobic amino acid valine at the sixth position. The carrier frequency ranges between 10% and 40% across equatorial Africa, decreasing to 1—2% on the North African coast and <1% in South Africa [15].

The highest frequency of sickle cell disease is found in tropical regions, particularly sub-Saharan Africa, India and the Middle-East [16]. Migration of substantial populations from these high prevalence areas to low prevalence countries in Europe and America has resulted in a dramatic increase of sickle cell disease in some European countries and the United States. In the US, the prevalence is approximately 1 in 5,000 affecting predominantly Americans of Sub-Saharan African descent [13]. In mainland France, ½ in 415 births is affected with SCD [17]. In. other areas like United Kingdom, 1 baby in every 2,000 is born with SCD; approximately 17% of the population in the Eastern province of Saudi Arabia carry the gene and about 1.2% have sickle cell disease [12]. Communities in Africa constitute a major part of the population that is vulnerable to many erythrocytic hereditary and hematological disorders such as haemoglobinopathies. The frequencies of abnormal haemoglobin variants vary from one population to another [15].

Blood is a complex fluid tissue responsible for the supply of oxygen and food to the other tissues of the body as well as removes carbon-dioxide and other waste products from the body. It is made up of cells suspended in a protein salt sqlution known as plasma. The cellular elements are erythrocytes, leucocytes and thrombocytes. The fluid plasma consists of various proteins such as thrombin, fibrinogen and globulin as well as electrolytes. The red cells are small, non-nucleated biconcave disc which contain haemoglobin. Dominant blood group genes express themselves through minute protein polysaccharide substances on the surface of the red cell membrane [18].

These substances are the product of specific genes at allelic-loci which are identical, the gene is said to be expressed in double dose, but when they are different or heterozygous, the gene is said to be expressed in single dose. Each particular gene is responsible for the production of antigen receptor on the red cell membrane. As humans differ in their facial and physical, so they differ in character. The genetic makeup of an individual is known as the genotype whereas the observable characteristic is known as the phenotype or blood group. The genotype is determined by the type of haemoglobin present on the red cells, while the phenotype is determined by the antigens present on the red cells, leucocytes and plat1ets or antibodies present on the plasma [18].

There are three basic types of persons when classified genetically; these are those who inherited the normal haemoglobin, those who inherited both the normal and abnormal and those who inherited only the abnormal. The inheritance of the abnormal haemoglobin in double dose as seen in sickle cell SS exposes one to various kinds of genetic diseases. The phenotypes are also inherited characteristics, the knowledge of which helps in compatible blood transfusion, compatible marital union and management of pregnancies resulting from incompatible marital union. The knowledge of blood group antigehs is equally applied in organ transplant, forensic and anthropologic investigation [19].

The frequency or distribution of genotype differs in places; some are most common in a certain area while others are rare. The phenotype and genotypes are identified in the laboratory using the properties nature have endowed them with, such as the antigen and antibody present on the surface of the red cells and antibodies in the plasma for the phenotype and charge present on the chromoprotein haemoglobin for genotype [20]. An unfortunate aspect of the anti-body-mediated immune response, as far as humans are concerned is that, any living material introduced into the body may be treated by the recipient as foreign; and antibodies will react against it. This may occur, for example, when bloods of the two individuals are not compatible the donor's red blood cells clump together in groups (agglutination) which may result in blockage of the recipient's blood vessels. The reason for this reaction is that the donor's red cells contain antigens which are complementary to antibodies present in the recipient's plasma. Unlike the normal immune response, the recipient does not actually produce antibodies in response to the donor's blood [18].

The entire human population can be divided into four blood groups on the basis of the reaction occurring between the bloods of different individuals when mixed together. These groups are called: A, B, AB and O. The capital letters stands for the type of antigens present in the person's red blood cells. The corresponding antibodies are carried in the plasma and can be represented by small letters: A, B, AB and O. Obviously if an individual has a particular antigen in his red cells he cannot have the corresponding antibody in his plasma, otherwise agglutination will occur. Thus, a person belonging to blood group A has red blood cells containing type-A antigens. The plasma of such individual will not contain type A antibodies but it does contain type B antibodies. A person of blood group B has red blood cells containing type-B antigens and type-A antibodies in the plasma. Group AB contains both antigens A and B and neither have antibodies. Group O has neither antigen but contain both antibodies [18].

In West African countries such as Ghana and Nigeria, the frequency of sickle cell trait is about 15% to 30%. Frequencies of the carrier state determine the prevalence of sickle-cell anaemia at birth. In Nigeria, 24% of the populations are carriers of the mutant gene and the prevalence of sickle-cell anaemia is about 20 per 1000 births. This means that in Nigeria alone, about 150, 000 children are born annually with sickle-cell anaemia, [21]. The high birth prevalence of SCD has highlighted the burden of SCD, such that in 2006, the World Health Organization (WHO) recognized SCD as a public health priority [22]. There• is limited information about the burden of SCD to the health system and the impact that it has on individuals. It is estimates that 50-80% of children born with SCD in Africa die before the age of 5 years [23] (Aygun and Odame, 2012). Although accurate statistics are often not available [24; 25] (Grosse *et al*., 2011; Serjeant and Ndugwa, 2003). Nigeria, which is the most populous sub-Saharan African country, has 150,000 newborns with sickle cell anaemia annually [22]. This accounts for half of the 300,000 babies born yearly with major haemoglobin disorders worldwide [22]. To the best of my knowledge there are no documented report on prevalence of sickle cell disease or any related awareness in many public school in Jalingo local government area of Taraba state, therefore this existing research on SCD and ABO blood group focuses on the awareness of the students of government day secondary school Howui Mile six Jalingo, aiming on how to reduce the prevalence of the disease. Therefore, this research aim at determining the prevalence of sickle cell disease among randomly selected students of government day secondary school (GDSS) Howui mile six, Jalingo, Taraba State.

2. Materials and Methods

2.1 Ethical approval

One hundred and thirty-nine (139) apparently healthy subjects (males-70, females-69) were randomly selected for this study. The institutional Ethical committee, as well as the principal and the Head of Medical Laboratory units of federal medical Center Jalingo approved the study. All the participants willingly donated their blood sample for the study. Their results were confidentially given to them.

2.2 Materials

The following materials were used for this study: 2mls syringe, Ethyline-diamine tetra-acectatic acid, dry cotton wool, 70 % prepared swab, biohazard box, tourniquet, hand glove. The above lists of the materials were used for samples collection. Antimonocolonal antigen, A, B and D, plain capillary tube, clean white tile, dry cotton wool. These listed materials were used for the determination of haemoglobin phenotype ABO blood grouping.

Cellular paper, commercially prepared buffer pH 8.6, filter paper, distilled water, 1ml Pasteur, washed container, Electrophoresis machine and dry cotton wool, were used for the determination of haemoglobin genotype.

2.3 Methods

2.3.1 Sample collection and preparation

2mls of vein puncture method was collected into EDTA container and inverted severally for proper mixing. About 1ml of the blood sample was washed three times using normal saline (0.85 % NaCI) to remove plasma proteins. The washed cells were re-suspended with equal volume of normal saline. The red cell suspension was mixed with equal volume of distilled water to lyse the blood cell. The resulting lysate was used for haemoglobin genotype determination [26].

2.3.2 ABO and Rh blood groups tests

Red cell phenotyping was carried out with standard Anti-sera to dictated antigens-antibodies reaction on the red blood cell. Blood obtained from vein puncture was used for each of the depression on the tile, with the used of Pasteur pipette three different spots of blood was placed on the tile, equal volume of anti-A(blue) was placed on the first drop and anti-B(yellow) to the second drop as well as ant-D(colorless) was placed on the last spot. Clean glass slide was used to mixed each spots respectively, it was rocked for about two minutes and agglutination were observed and result was interpreted. For Rhesus D typing, a drop of anti-D serum (Atlas Medical Cambridge, UK) was placed in a clean labeled test tube and a drop of control placed in a second tube. 1 drop of 5% RBC suspension in saline was then added and incubated at 37°C for 30mins. At the end of the incubation period, the contents of the tube were mixed gently and centrifuged for 30 seconds at 1000g. Agglutination was read macroscopically and microscopically in doubtful cases. All negative results were confirmed using the indirect antiglobulin test (TAT) procedure (also for confirmation of weak D).

2.3.3 Blood genotype test

Haemoglobin Electrophoresis

Principle

Different types of haemoglobin (Hb) have different migration rate because Hbs are proteins. They can be made to run to either anode or cathode depending on the charge determines the charge carried by the Haemoglobin (Hb). Here alkaline cellulose acetate is used in an alkaline buffer at pH 8.4; this tends to make the Hb to migrate to the anode where there is negative charge.

Cellulose acetate electrophoresis technique as used to determine haemoglobin genotype. The method described by Brown was used for haemoglobin electrophoresis. A small quantity of haemolysate of venous blood from each of the subjects was placed on the cellulose acetate membrane and carefully introduced into the electrophoretic tank containing Tris-EDTA-Borate buffer at pH 8.6. The electrophoresis was then allowed to run for 15 - 20 minutes at an electro

motive force (emf) of 160 V. The results were read immediately. Haemolysates from blood samples of known haemoglobin (AA, AS, SS) were run as control [27].

3. Results

Table 1: Distribution of haemoglobin genotype of randomly selected students of GDSS Howui, Jalingo, Taraba State.

Haemoglobin genotype	Frequency	Percentage (%)
HbAA	106	76.26
HbAS	32	23.02
HbSS	1	0.72
Total	139	100

The result for the genotype showed that, among the 139 subjects screened for the haemoglobin, about 76.26 % have HbAA genotype, 23.02 % belong to HbAS genotyype and about 0.72 belong to 1-IbSS % genotype (table l). The result for blood groupings showed that thirty-five subjects *(25.18* %) belong to blood group A, thirty subjects (21.58 %) belong to blood group B. Also, eight subjects *(5.76* %) have blood group AB and fifty-five subjects (39.57 %) belong to blood group O (table 2). The number of Rhesus negative where as follows: four subjects (2.87 %) belong to blood group B, two subjects (1.44 %) belong to blood group AW. Five subjects (3.59 %) belong to blood group 0 and none belong to group K (table 2). The result revealed that, one hundred and twenty-eight subjects are Rhesus positive (+) and only eleven subjects are Rhesus negative (+) (table 3). By comparison blood group O positive have the highest frequency of normal haemoglobin HbAA 35 (25.18 %) and also highest number of subjects with abnormal haemoglobin HbAS 15 (10.79 *%).* The result showed one subject with O blood group and having the sickle cell disease HbSS (table 4). The result further showed blood group B with frequency of 20 (14.39 %) subjects having normal 1-IbAA and eight (5.76 %) with abnormal haemoglobin HbAS. The next in this order is blood group A with the occurrence of 18 (12.95 %) having normal HbAA and four (2.87 %) with abnormal haemoglobin HbAS (table 4). Blood groups AB, B and 0 all have four (2.87 %) subjects with normal HbAA and one HbAS each except AB having two HbAS haemoglobin. Aff has one subject with normal haemoglobin and none with abnormal haemoglobin (table 4).

Table 2: Distribution of ABO blood group of some selected students of GDSS Howui, Jalingo, Taraba State.

Haemoglobin phenotype Rh 'D' positive	Frequency	Percentage (%)
A^+	35	25.18
B^+	30	21.58
AB^+	8	5.76
O^+	55	39.57
Haemoglobin phenotype Rh 'D' negative		
A^-	0	0%
B^-	4	2. 87
AB^-	2	1.44
O^-	5	3.59
Total	139	100

Table 3: Rhesus D positive and negative blood group distribution by % amongst the study subjects

Rhesus (D) group Percentage (%)	Frequency
Negative 7.91	11
Positive 92.09	128

Table 4: Distribution of haemoglobin genotype and ABO blood group of some selected students of GDSS Howui, Jalingo, Taraba State

ABO Blood group	Hb AA	Hb AS	Hb SS
A^+	18 (12.95 %)	4 (2.87 *%)*	0 (0.0 %)
B^+	20 (14.39 *%)*	8 *(5.76 %)*	0 (0.0 %)
AB^+	4 (2,87 %)	2 (1.44 *%)*	0 (0.0 *%)*
0^+	35(25.18 *%)*	15 (10,79 %)	1 (0.72%)
A^-	3 (2.16%)	1(0.72%)	0(0.0%)
B^-	4 (2.87 %)	1 (0.72 %)	0 (0.0 %)
AB^-	1 (0.72 %)	0 (0.0 %)	0 (0.0 %)
O^-	4 (2.78 %)	1 (0.72 %)	0 (0.0 %)

4. Discussion

Haemoglobin genotypes and blood groups are all inherited blood characters. The inherited disorders of haemoglobin are the most common gene disorders with 7% of the world population being carriers [11]. In Nigeria, the distribution of HbSS was given as follows: Lagos 1.3 %, Ogbomoso Oyo State 3.0 %, University students, Port Harcourt, River State 1.5 %, Niger Delta University students 1.3 % [28]. The distribution of AS was reported as follows: black Americans 8 — 16 %, for white Americans 8 — 10 %, Europeans 15 % 1%—15%, for Caribbean's 3%—8%, Africans 40.5 %. In Nigerians the distribution of AS is as follows: Lagos 26 %, Ogbomoso, Oyo State 21.0 %, University students, Port Harcourt, River State 29.4 %, Niger Delta University students Welberforce Island, Amassoma, Bayelsa State 26.0 % [29].

In this study, the frequency of HbAA was 76.26 % while HbAS was found to be 23.02% and HbSS 0.72 % among the selected subjects. This study is similar to previous study carried out among undergraduate students of Hassan Usman Katsina Polytechnic, Katsina state [30]. In Nigeria distribution rates for HbSS of 1.3% and 1.5%, for Lagos and Rivers states respectively were observed. The low prevalence of HbSS in the study population could be attributed to increased awareness of the disease, improved socio-economic conditions and other environmental and genetic factor which have an overall effect on the sickling gene pool. It is equally possible that the Hardy - Weinberg equilibrium (Aspect of human genetic second edition) must have been disturbed which has led to more people acquiring normal haemoglohin gene and sickle cell trait while the homozygous sickle cell gene is gradually tending to zero. The observed frequency of 76.26 % for HbAA (table 1) is slightly above the normal range of 55-75 % as earlier reported for Blacks [31]. The frequency of HbAS 23.02 % is within the normal range of 20- 30 % reported for Nigerians and 20-40 % in Africa generally [2]. The number of people with homozygous SS in Nigeria is still high [22]. This is thought to be due to the absence of carrier testing programs or premarital counseling/testing for prospective couples prior to marriage in a bid to reduce the prevalence of haernoglobin disorders. The universal neonatal screening program is an effective way to diagnosis the presence of

haemoglobinopathies. Experience in Belgium has shown universal neonatal screening to be an excellent health education tool. Countries in Africa can benefit by implementing similar programs, as their development is pivotal to improving the health care of those affected by haemoglobin disorders.

The frequency of ABO blood groups varies from race to race. Among Western Europeans, 42 % have group A, 9 % group B, 3 % group AB and the remaining 46 % group 0. However, some Eastern Europeans have a higher proportion, up to 40 % of group B blood while pure native American Indian belong exclusively to blood group 0 [32], American blacks generally have frequencies of A, B, AB and 0 blood groups of 27 %, 20 %, 4 % and 49 % respectively. In Nigeria, few published works on the frequencies of the ABO blood groups among ethnic groups/tribes were reported. The first was that of [33] who reported the frequencies among Yoruba and Hausa as follows: 21 % for group A, 17 % for group B, 2 % for group AB and 58 % for group 0. Many previous reports [34, 35, 30] are in agreement with the frequencies obtained in this study and it suggest that group 0 appears to show predominance over the other blood groups. However, an exception to this study is the Gwari tribe of Abuja and the Rubuka tribe of Plateau state in Nigeria, where group B has been reported to show predominance over the other blood groups in those states [36]. The high frequency of group 0 in this population provides an advantage in terms of availability of blood for blood transfusion especially in emergencies. However, some level of caution has to be exercised since some group 0 blood is known to contain potent immune haemolytic antibodies (haemolysins). Routine haemolysin test on every group 0 blood should be encouraged to reduce the risk of transfusion reaction [18].

The frequency of Rhesus 'D' positive antigen in this study was 92.09%. This falls within the range of 90 - 96.7 % as [37, 38] recorded the same pattern of ABO blood groups differ in populations and Ethnic groups.

In this study, sex, age and disease conditions were not considered critical; rather the results depended on the genetic constitution of the subjects. However, the distribution of the blood groups and haemoglobin genotypes among different age groups were analysed. It was discovered that majority of the students fell into the 13 — 25 years group and in this group; the blood groups were better distributed than other age groups. Most of the people with HbAA were within 13 — 20 years. There was no test of significance between groups so it is difficult to state statistically whether age has any influence on these inherited characters.

5. Conclusion

The knowledge of the various abnormal haemoglobin variants is vital in the safe and effective transfusion services in this locality as well as in civic registration and forensic medicine. It will also be useful in genetic counseling of prospective couples to make informed decisions aimed at reducing the sickling gene pool of this ethnic nationality and in Nigeria.

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