The Estimation of the Frequency of Abo/Rhesus Blood Groups and Sickle Cell Trait of Patients Visiting KNUST Hospital Laboratory for Routine Hematological Examination

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Abstract

The ABO/Rhesus blood and Sickle cell trait are genetically determined and not acquired. Knowledge of the ABO/Rhesus blood groups is important in blood transfusion, organ transplantation, etc. while that of sickle cell trait is helpful in combating the sickle cell disease. The study was conducted at the Kwame Nkrumah University of Science and Technology (KNUST) hospital to determine (primary data) and therefore update (secondary data) the prevalence of the ABO/Rhesus blood groups and the sickle cell trait among the students and staff of KNUST and the surrounding towns. This study also provided a possible projection of the ABO/Rhesus blood groups and the Sickle cell trait among the Ethnic groups. In all 700 samples (primary data) were collected from patients visiting the laboratory for routine hematological examination over a period of 5 months from October, 2009 to February, 2010. These data compared with and updated the secondary data which consisted of 2637 sample examined for the blood grouping and 3053 samples examined for the sickle cell trait previously. The subjects comprised of both males and females including infants with exception of the secondary data which comprised of only females. The blood groups were determined by agglutination method while the sickle cell trait was determined by Sodium metabisulphite method. Blood group O had the highest frequency in both the primary and secondary data recording 52.8% and 51.3% respectively while blood group AB was the least occurring in both data with 5.3% and 3.7% respectively. Blood groups A and B were comparable in both data sets with A constituting 21% and 22.7% respectively and B recording 20.9% in primary data and 22.3% in secondary data in the secondary data. The frequency for the sickle cell trait was 18.4% positive and 81.6% negative in the primary data and 11.3% positive and 88.7% negative in the secondary data. Among the ethnic groups, 482 Akans, 108 Mole-Dagbanes and less than 100 of the others were sampled (primary data). Blood group O was the most abundant in all the ethnic groups ranging from 56% - 35.7% with the exception of the Guan which had blood group B to be most common with 42.9%. Again, blood groups A and B occurred in approximately equal proportions while blood group AB was the least common. The Rhesus and Sickle cell trait also followed a similar pattern as in both the primary and secondary data. The distribution of the ABO blood group system followed a welldefined, predictable pattern in both data sets and among the ethnic groups with groups O and AB having the highest and lowest frequencies respectively. Rhesus trait and the Sickle cell traits were abundant in the population. Therefore urgent need for vaccine against erythroblastosis fetalis and premarital genetic counseling are recommended.

Keywords: Sickle Cell, Trait of Patients, Abo/Rhesus Blood Groups, & Routine Hematological Examination

I. INTRODUCTION

When blood transfusions were first attempted, illness and even death sometimes resulted. Eventually, it was discovered that only certain types of blood are compatible because red blood cell membranes carry substances that are antigenic to blood recipients (Mader, 1997). Advance knowledge of the blood, its constituents, and the shape of the red blood cells as well the discovery of the different blood groups especially that of the ABO/Rhesus blood groups have solved many health complications such as Erythroblastosis fetalis, agglutination during blood transfusion and organ transplanting etc. Having knowledge of a person's blood led to the discovery of the sickle cell trait which for many years had resulted in early death of its homozygous bearers. The frequencies of the ABO/Rhesus blood groups and the sickle cell condition change with time, therefore the need for periodic assessment of their occurrence. For instance in the KNUST Hospital, it was only in 2004 that another student under the supervision of Dr Kofi Owusu-Daaku assessed the prevalence of these parameters. Thus the frequencies of the ABO/Rhesus blood groups and sickle cell condition in Ghana are well not known. Limited data available are only on the association of these to some ethnic groups (Acquaye, 2004). Therefore the need for this work.

II. LITERATURE REVIEW

The prevalence of the ABO/Rhesus blood groups and the sickle cell condition differ in different countries and among different ethnic groups within the same country. Even within countries and ethnic groups the prevalence of these parameters changes with time due to non-random mating, migration etc. In some cases, for example, the prevalence of the sickle cell trait is consciously regulated which results in its decrease in the advance countries while in most developing countries there are still increased prevalence (Ohene – frempong, 2001). In many countries the blood group O is known to be more common than A and B which may either have equal frequencies or A occurring more than B. The AB blood group is the least prevalent. Blood group B has its highest frequency in Northern India and neighboring Central Asia, with its occurrence diminishing both towards the west and the east, falling to single digit percentages in Spain (Transfusion Division, United States Army Medical Research Laboratory, 1971). Blood group A is associated with high frequencies in Europe, especially in Scandinavia and Central Europe, although its highest frequencies occur in some Australian Aborigine populations and the Blackfoot Indians of Montana (Dean, 2005).

In Africa not much work has been done on the ABO/Rhesus blood groups. So far there is no African country with it ABO/Rhesus blood groups of their entire country known (http://www.bloodbook.com/world-abo.html), however few countries have on record the ABO/Rhesus blood groups of some of their states, metropolis and ethnic groups. For instance in the Kano metropolis of Nigeria the blood group O had the highest distribution of 55.3%, followed by similar result of 20.8% and 207% of blood groups A and B respectively while blood group AB had the lowest distribution of 3.2%. The prevalence of Rh (D) negative subjects amongst the population studied was 5.1%. Similar result were obtained when the distribution of A Rh (D) positive, A Rh (D) negative, B Rh (D) positive, B Rh(D) negative, O Rh(D)positive and O Rh (D)negative(20.1%, 1.3%, 19.6%, 1.1%, 50% and 2.3% respectively) of pregnant women were compared to 19.6%, 0.83%, 19.7%, 1.0%, 53.9%, and 3.% respectively of blood donor (Imoru *et al.*, 2003). Again in Ogbomoso, Oyo State, Nigeria the prevalence of blood group O were 50%, blood group A were 22.9%, blood group B were 21.3% and blood group AB were 5.9% (Bakare *et al.*, 2005). In the Akan ethnic group of Ghana, the blood group O is prevalent with frequency ranging from 50% to 57% (Acquaye, 2004). Lack of knowledge on the sickle cell trait has led to many deaths and disability in people who associate the condition with superstition. (http://www.ghanaweb.com/GhanaHomePage/tribes/ewe)

Recent studies have shown that some diseases are more associated with particular blood groups than others. Therefore knowing the dominant blood group within a particular population can give an indication of the disease that is likely to prevail in such a population and therefore how to treat it. The blood group of an individual is for a life time and is only under very rare circumstances that we have a one's blood group changing (Hovinga, 2007). A case in point is the report of an international team of researchers led by Henrick Clausen of the University of Copenhagen, Denmark who discovered a bacterial enzyme that can convert red blood cells of types A, B and AB into O by stripping away their identifying surface antigens (http://www.Anthro.palomar.edu/blood/ABO_system/htm, 2009). Another way by which an individual's blood group can change is by bone marrow transplant. When an individual receives a bone marrow from someone of different ABO type (for example, B type patient receives a type O bone marrow) the patient's blood type will eventually convert to the donor's type, i.e. O. The possibility of changing an individual's blood group has a potential for rendering previously incompatible blood groups compatible.

A. Blood Group

A blood group or blood type is a classification of the blood based on the presence or absence of inherited antigens on the surface of the red blood cells. More than 35 blood groups are known (Seeley *et al.*, 1998). The international society of Blood Transfusion (ISBT) recognizes a total of 30 human blood groups. These include the ABO, Rhesus, Kell, Duffy, Lutheran, etc. These blood groups are inherited in a mendalian fashion. These antigens may be carbohydrate, protein, glycoprotein or glycolipids .The type of antigen (A and / or B) attached to a red blood cell is a consequence of the action of a glycosyltransferase enzyme that adds specific sugars to precursor substances on the surface of the red blood cells (Technical Manual of American Association of Blood Bank, 1996). Over 600 different blood group antigens have been identified (American Red Cross Blood Services, 2001). However many of these antigens are very

rare or are mainly found in certain ethnic groups. Several of the red blood cell surface antigens collectively forming a blood group system stem from one allele (Maton *et al*, 1993). Some of these antigens on the red cell's surface are also known to be present on the surface of other cell types of various tissues and this explains why in cases of tissue and organ transplant both the recipient and the donor are checked for compatibility.

a. Abo Blood Groups

The ABO blood group system is the most important blood system clinically especially in human blood transfusion. Grouping of the blood in ABO system is based on the presence or absence of one or both antigens A and B. There are four types of ABO blood groups: blood groups A, B, AB and O. Blood groups A and B have antigens A and B on the surface of the red blood cells respectively. While the group AB has both antigen A and B, group O has neither antigen A nor B (Seeley *et al*, 1998). The ABO blood group system is widely credited to have been discovered by the Austrian scientist Karl Landsteiner, who found three different blood types in 1900 (Landsteiner, 1900). Due to inadequate communication at the time it was subsequently found that Czech serologist Jan Janský had independently pioneered the classification of human blood into four groups (Jansky, 1901), but Landsteiner's independent discovery had been accepted by the scientific world while Janský's remained in relative obscurity. Janský's classification is however still used in Russia. Landsteiner described A, B, and O; Decastello and Sturli discovered the fourth type, AB, in 1902 (Decastello *et al*, 1902). Ludwik Hirszfeld and E. von Dungern discovered the heritability of ABO blood groups in 1910–11, with Felix Bernstein demonstrating the correct blood group inheritance pattern of multiple alleles at one locus in 1924 (Crown, 1993).

b. Transfusion reaction

Due to the antigen-antibody reaction (agglutination) that normally leads to blood clotting and death when a donor and a recipient's bloods are incompatible, bloods are constantly screened for compatibility before transfusion. Antibodies formed against the antigens on the red blood cell surface are responsible for this agglutination. These antibodies (Anti-A antibodies and Anti-B antibodies) are usually immunoglobulin M (IgM) antibodies which are usually produced in the first years of life by sensitization to environmental substances such as food, bacteria, and viruses. As a result of the agglutination only certain blood types are compatible, as summarized in the table below:

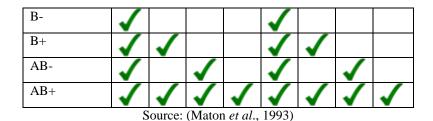
Recipient	Donor			
Blood group A	A or O			
Blood group B	B or O			
Blood group AB	A, B, AB or O			
Blood group O	0			

Table 1 Compatible blood group.

Source: (Maton et al., 1993)

Table 2 The ABO/Rhesus bloba compatibility.								
RECIPIENT	DONOR							
	0-	0+	A-	A+	B-	B+	AB-	AB+
0-	\checkmark							
0+	>	>						
A-	\checkmark		\checkmark					
A+	\checkmark	\checkmark	\checkmark	\checkmark				

Table 2 The ABO/Rhesus blood compatibility.



c. Hemolytic disease of the new born

The hemolytic disease of the new born is caused by incompatibility between the mother's and fetus blood group. This result in hemolysis (breaking) of the fetus red blood cell and rendering the fetus anemic when born or even dead. The ABO blood group incompatibility between the mother and child does not usually cause hemolytic disease of the newborn because antibodies to ABO blood groups are usually of the IgM type which do not cross the placenta. However, in an O- type mother, IgG ABO antibodies are produced and the baby can develop ABO hemolytic disease of the newborn.

d. Blood and diseases

In the last 20 years there has been increasing evidence that blood groups have function and play a biological role. Antigens identified on red blood cells, which define specific blood groups, are known to be important as receptors and ligands for bacteria, parasites and immunological important proteins, e.g. those associated with movement of normal and malignant cells throughout the body (Garratty, 2000). The result is an association of certain diseases with particular blood groups, as shown the table below.

1	V 1
	DOMINANT
	BLOOD
DISEASES	GROUP
Schizophrenia	0
Plague	0
Cholera	0
Leprosy	В
Tuberculosis	O, B
Streptococcus pneumonia infection	В
Gonorrhea	В
Escherichia coli infection	B, AB
Salmonella infection	A, AB
Smallpox	A, AB
Mumps	0
Duodenal ulcer	0
Pernicious anemia	А
Paralytic poliomyelitis	0
Sources (Muschel 1066, Come	try 2000 .)

Table 3: Some diseases known to be associated with particular blood types of the ABO blood group system

Source: (Muschel, 1966; Garratty, 2000;)

B. Rhesus Factor (Rhesus Blood Groups)

The Rhesus system is named after Rhesus Macaque, following experiments by Karl Landsteiner and Alexander S. Wiener, which showed that rabbits immunized with rhesus monkey red cells, produced an antibodies that agglutinate the red blood cells of many humans. Landsteiner and Alexander S. Wiener discovered this factor in 1937 (Landsteiner, 1940). The Rhesus system is the second most significant blood-group system in human-blood transfusion. The most significant Rhesus antigen is the Rhesus D (RhD) antigen because it is the most immunogenic of the five main rhesus antigens. The terms "positive" or "negative" refer to either the presence or absence of the RhD antigen irrespective of the presence or absence of the other antigens of the Rhesus system. It is common for RhD-negative individuals not to have any anti-RhD IgG or IgM antibodies, because anti-RhD antibodies are not usually produced by sensitization against environmental substances. However, RhD-negative individuals can produce IgG anti-RhD antibodies following a sensitizing event: possibly a fetomaternal transfusion of blood from a fetus in pregnancy or occasionally a blood transfusion with RhD positive RBCs (Talaro *et al*, 2005).

a. Rhesus antigens

The proteins which carry the Rhesus antigens are transmembrane proteins, whose structure suggests that they are ion channels (Talaro *et al.*, 2005). The main antigens are C, D, E, c and e, which are encoded by two adjacent gene loci, the RHD gene which encodes the D antigen (Landsteiner et al, 1940) and the RHCE gene which encodes both the C and E antigens (Wsutoday Test for Rh factor). There is no recessive d antigen. "d" indicates the absence of the D antigen (the gene is usually deleted or otherwise non-functional).

b. Inheritance of the rhesus trait

The Rhesus blood groups are also inherited in a Mendalian fashion

Genotype	symbol	Rh(D) status			
cde/cde	Rr	Homozygous negative			
CDe/cde	R1r	Heterozygous Positive			
CDe/Cde	R1R1	Homozygous Positive			
cDE/cde	R2r	Heterozygous Positive			
CDe/Cde	R1R2	Homozygous Positive			
cDE/cDE	R2R2	Homozygous Positive			

Table 4: Genotype and the corresponding phenotypes of the Rhesus blood groups

c. Erythroblastosis fetalis

Though the term erythroblastosis refers to the presence of immature red blood cells and fetalis refers to a fetus, erythroblastosis fetalis is the destruction of the red blood cells of the fetus as a result of rhesus D antigen incompatibility between the mother and the fetus (Encyclopedia Britannica Library, 2008). The condition is also known as the Hemolytic disease of the newborn (often called Rhesus or Rh disease). This occurs when a rhesus negative mother carries a rhesus positive fetus. The Rh D antigen stimulates the production of maternal RhD antibodies leading to the destruction of the fetal red blood cells. This usually happens after the first pregnancy because not enough RhD antibodies will have been produced to abort this fetus. The vast majority of Rh disease is preventable in modern antenatal care by injections of IgG anti-D antibodies (Rho (D) Immune Globulin). The incidence of Rhesus disease is mathematically related to the frequency of RhD negative individuals in a population, so Rhesus disease is rare in East

Asians, South Americans, and Africans, but more common in Caucasians. About 85% of white people and 88% of Black people in the United States are Rhesus positive (Seeley, 1998)

Country	O+	A+	B+	AB+	0-	A-	B-	AB-
Austral	40%	31%	8%	2%	9%	7%	2%	1%
Austria	30%	33%	12%	6%	7%	8%	3%	1%
Belgium	38%	34%	8.50%	4.10%	7%	6%	1.50%	0.80%
Brazil	36%	34%	8%	2.50%	9%	8%	2%	0.50%
Canada	39%	36%	7.60%	2.50%	7%	6%	1.40%	0.50%
Denmark	35%	37%	8%	4%	6%	7%	2%	1%
Estonia	30%	31%	20%	6%	4.50%	4.50%	3%	1%
Finland	27%	38%	15%	7%	4%	6%	2%	1%
France	36%	37%	9%	3%	6%	7%	1%	1%
Germany	35%	37%	9%	4%	6%	6%	2%	1%
Hong Kong SAR	40%	26%	27%	7%	0.31%	0.19%	0.14%	0.05%
Iceland	47.60%	26.40%	9.30%	1.60%	8.40%	4.60%	1.70%	0.40%
India	36.50%	22.10%	30.90%	6.40%	2.00%	0.80%	1.10%	0.20%
Ireland	47%	26%	9%	2%	8%	5%	2%	1%
Israel	32%	34%	17%	7%	3%	4%	2%	1%
New Zealand	38%	32%	9%	3%	9%	6%	2%	1%
Norway	34%	42.50%	6.80%	3.40%	6%	7.50%	1.20%	0.60%
Poland	31%	32%	15%	7%	6%	6%	2%	1%
Portugal	36.20%	39.80%	6.60%	2.90%	6.00%	6.60%	1.10%	0.50%
Saudi Arabia	48%	24%	17%	4%	4%	2%	1%	0.23%
Spain	36%	34%	8%	2.50%	9%	8%	2%	0.50%
Sweden	32%	37%	10%	5%	6%	7%	2%	1%
Netherlands	39.50%	35%	6.70%	2.50%	7.50%	7%	1.30%	0.50%
Turkey	29.80%	37.80%	14.20%	7.20%	3.90%	4.70%	1.60%	0.80%
United Kingdom	37%	35%	8%	3%	7%	7%	2%	1%
United States	37.40%	35.70%	8.50%	3.40%	6.60%	6.30%	1.50%	0.60%
Mean	36.50%	33.40%	11.90%	4.20%	6.10%	5.70%	1.70%	0.70%
Std dev	5.30%	5.00%	6.20%	1.90%	2.20%	2.10%	0.60%	0.30%

Table 5: shows the distribution the ABO/Rhesus blood groups in various countries.

Source: (http://www.bloodbook.com/world-abo.html).

C. Hemoglobin Disorders

Hemoglobin disorders are heritable blood condition that affects how oxygen is carried on the red blood cells. The disorder falls into two main categories: thalassaemias and sickle – cell disease. It is estimated that each year over 300,000 babies with severe forms of these diseases are born worldwide; majority being in the low and middle income countries. Approximately 5% of the world's populations are carrier of the gene for sickle – disease or thalassaemias. In some regions the proportion of the people who are carriers of the gene is as high as 25% (WHO, 2009).

a. Thalassaemia

People with thalassaemias are unable to produce enough haemoglobin. Less haemoglobin in the red blood cells means less oxygen for the cells, tissues and organs, this may result in cell, tissue or organ damage. There are two main types of thalassaemias, Alpha and Beta thalassaemias based on the protein chain that make up the normal haemoglobin. Alpha and Beta thalassaemias have both mild and severe forms. Both forms of the disease are common in Asia, the Mediterranean basin and the Middle East. Thalassaemias require regular blood transfusions to maintain a healthy supply of haemoglobin and sustain life. As a result of multiple transfusions, organs become severely overloaded with

iron and secondary treatment is needed to manage this condition. Thalassaemias can be cured by successful bonemarrow transplantation; however this procedure is expensive and not readily available in most settings. (WHO, 2009)

b. The Sickle Cell Condition

The sickle-cell trait is a heritable blood disorder that arises from a single amino acid substitution in one of the component proteins of hemoglobin. The affected component protein (globin), becomes defective. Hemoglobin molecules constructed with such proteins have a tendency to stick to one another, forming strands of hemoglobin within the red blood cells. The cells that contain these strands become stiff and assume the shape of a sickle (Plate A) (http://www.google.com/sickle-cell disease).

Plate A: Shapes of normal and sickle cell red blood cells

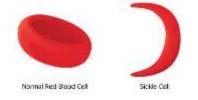


Image source: from http://www.kidhealth.com, 2009.

Sickled red blood cells die much more rapidly than normal red blood cells. The life span of normal red blood cells is approximately 120 days in the bloodstream but sickle cells last for only 10-12 days. The body cannot create replacements fast enough and anemia develops due to the chronic shortage of red blood cells. Further complications arise because sickle cells do not fit well through small blood vessels, and can become trapped. The trapped sickle cells form blockages that prevent oxygenated blood from reaching affected tissues and organs. Considerable pain results in addition to damage to the tissues and organs. This damage can lead to serious complications, including stroke and an impaired immune system (Mannino, 1995)

c. Inheritance of the sickle cell trait

The sickle cell trait is the result of a mutation in which the amino acid glutamic acid is substituted by value in the alpha chain of the amino acid sequence in the globin component of haemoglobin. This changes the haemoglobin from the normal (Haemoglobin A) to the abnormal (Haemoglobin S). The trait is inherited in a Mendalian fashion with only the homozygous recessive genotype (SS) suffering from the condition. Generally, heterozygous carriers (AS) do not suffer from the disease, though they can transmit the trait to their progeny.

d. Symptoms/Complications

Common symptoms of the sickle cell disease include fatigue, paleness, and a shortness of breath. A particularly severe form of anemia - aplastic anemia, occurs following infection with parvovirus. Parvovirus causes extensive destruction of the bone marrow, bringing production of new red blood cells to a halt. Bone marrow production resumes after 7-10 days; however, given the short lives of sickle cells, even a brief shut-down in red blood cell production can cause a precipitous decline in hemoglobin concentrations. This is called "aplastic crisis." Painful crises, also known as vasoocclusive crises, are a primary symptom of sickle-cell anemia in children and adults. The pain may be caused by small blood vessel blockages that prevent oxygen from reaching tissues. A bone pain, may occur when blood is shunted away from the bone marrow though this could be by through some other mechanism other than blockage by sickle cells. These crises are unpredictable, and can affect any area of the body, although the chest, abdomen, and bones are frequently affected sites. There is some evidence that cold temperatures or infection can trigger a painful crisis, but most crises occur for unknown reasons. The frequency and duration of the pain can vary tremendously. Crises may be separated by more than a year or possibly only by weeks, and they can last from hours to weeks. The hand-foot syndrome is a particular type of painful crisis, and is often the first sign of sickle-cell anemia in an infant. Common symptoms include pain and swelling in the hands and feet, possibly accompanied by a fever. Hand-foot syndrome typically occurs only during the first four years of life, with the greatest incidence at one year. Sickle cells can impede blood flow through the spleen and cause organ damage. In infants and young children, the spleen is usually enlarged. After repeated incidence of blood vessel blockage, the spleen usually atrophies by late childhood. Damage to the

spleen can have a negative impact on the immune system, leaving individuals with sickle-cell anemia more vulnerable to infections. Infants and young children are particularly prone to life-threatening infections (FAQ'S, 2007)

e. The sickle cell trait and malaria

The areas of the world associated with the sickle cell trait are also strongly affected by malaria. It is thought that the genetic mutation associated with the sickle cell trait occurred thousands of years ago. Coincidentally, this mutation increased the likelihood that carriers would survive malaria outbreaks. Survivors then passed the mutation on to their offspring, and the trait became established throughout areas where malaria was common. The severity of the symptoms cannot be predicted based solely on the genetic inheritance. Some individuals develop health or life-threatening problems in infancy, but others may have only mild symptoms throughout their lives. For example, genetic factors, such as the continued production of fetal hemoglobin after birth, can modify the course of the disease. Fetal hemoglobin contains gamma-globin in place of beta-globin; if enough of it is produced, the potential interactions between hemoglobin S molecules are reduced.

f. Frequency of the sickle cell trait

The Sickle cell trait is common among people whose ancestors come from sub-Saharan Africa, South America, Cuba, Central America, Saudi Arabia, India, and the Mediterranean countries such as Turkey, Sicily, Greece, and Italy. Once thought to be a rare disease, research now shows that in the United States, sickle cell condition occurs in about 1 in every 500 African-American children and in 1 in every 900 Hispanic American children (American Sickle Cell Anemia Association, 2009). In Ghana for instance two percent of babies carry the condition each year. This contrast sharply with the 0.025% in the United States. The figures for Ghana are similar to those in west and central Africa where the disease is most prevalent. Whereas 95% of American children with the disease survive beyond age 20, only 5% of the Ghanaian children live to see their fifth birthday. The reasons for this include ignorance and lack of timely diagnosis of the disease. Even where knowledge exists, facilities and resources may be Inadequate or lacking (Ohene-frempong, 2001)

III. MATERIALS & METHODS

A. Study site and Sample size

The study was carried out at the KNUST hospital in Kumasi from October 2009 to February 2010 (primary data) and January 2009 to December 2009 (secondary data). 700 patients were recruited in the study. Primary data collected from these patients were analyzed with secondary data previously collected from 2637 patients for blood group examination and 3053 patients for the sickle cell examination. The study lasted for a period of 5 months. The data were statistically analyzed using one – way ANOVA.

B. Subject selection

The sample comprised of patients of all ages and sexes ranging from infants to adults. Anybody who came to the laboratory for haematological examination as at the time we were present was included in our sample. Most of the subjects were adult with few infants. The subjects for the primary data were made up of students, ante-natal patients, etc. while all the subjects for the secondary data were ante-natal patients only.

C. Sample collection

Precautions were taken to ensure that the site to be punctured was disinfected with ethanol to avoid introducing microbes into the wound. The punctured site was covered with a cotton wool or gauze to stop any bleeding. The samples were taken either by venepuncture or capillary method depending on the type of examination the patients was undertaking. The venous blood was taken into a tube containing ethylenediamine tetraacetic solution (EDTA) or other anticoagulant. A tourniquet was placed around the upper arm to apply pressure thus restricting blood flow through the vein .This constricts the blood vessels below the tourniquet resulting in accumulation of blood which is taken. The tourniquet was removed once the blood was collected to restore circulation. In the capillary method, the thumb was punctured with a lancet and the blood oozing out following a gentle pressure was collected.

D. Questionnaires

Questionnaires were administered to the patients during the sampling of the primary data on the basis of acquiring more information in regards to their ethnicity. This will enabled us to make a possible projection for the distribution of the various ABO/Rhesus blood groups and the Sickle cell traits among the various ethnic groups in Ghana. The ethnic groups considered were based on the five main ethnic groups generally recognized in Ghana. The ethnic groups are further divided into subgroups based on some similarities in their languages. The five major ethnic groups Akan, Ewe, Ga-Adangbe, Guan and Mole – Dagbane.

AKAN

The Akan ethnic group is further subdivided into Asante, Fante, Akuapim, Akwamu, Akyem, Wassa, Denkyira, Ahanta, Bono, Nzema, Kwahu and Safwi.

EWE

The subgroups within the Ewe ethnic group are Nkonga, Tafi, Logba, Sontrokofi, Lolobi, Likpe 1.

GA-ADANGBE

The Ga- Adangbe consists of the Ga, Adangbe, Ada, Kroboo, and Kololi.

<u>GUAN</u>

Larteh-Kyerepong of the Eastern region and Anum-Boso forms the subgroups of the Guans.

MOLE-DAGBANE

This is made up of those in the north, upper east and upper west. They include Dagomba, Nanumba, the Mossi and Mamprusi.

Source: Encyclopedia Britannica, 2008

E. Determinantion of Abo/Rhesus Blood Group

a. Tile technique

Three drops of the test blood, about 50μ l each, labeled A, B and D were placed about three centimeters from each other on a tile to indicate where anti A, anti B, and anti D sera were to be added respectively. One drop of anti A serum is put on the blood sample A, one drop of anti B serum into sample B and one drop of anti D serum into sample D. Each sample was well mixed and swirled gently until agglutination occurs or otherwise. The determination of the blood group was as follows:

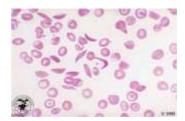
- Agglutination in sample A means blood group was A
- Agglutination in sample B means blood group was B
- Agglutination in both samples A and B means blood group was AB
- No agglutination in either sample A or B means blood group was O
- Agglutination in sample D means blood group was rhesus positive
- No agglutination in sample D means blood group was rhesus negative

F. Determination of Sickle Cell Trait

a. The sodium metabisulphite method for sickle cells

The sickling fluid was first prepared dissolving 0.2g of sodium metabisulphite in 10mls of distilled water. The blood sample (50µ1) was taken onto a clean slide. A drop of the sickling fluid was added onto the blood and well mixed to absorb oxygen thereby causing the red blood cells with the sickle cell trait to assume the sickle shape. A clean coverslip was then placed on the mixture. The mixture was blotted to remove any superfluous blood. An hour period was allowed for maximum oxygen absorption and assumption of the sickle shape by the sickle cells. The slide was then examined under the microscope for the sickle cells using X40 magnification. The results were recorded as positive or negative depending on whether sickle cells were seen or not.

PLATE B: Showing the sickle and normal red blood cells under the microscope



Source: http://www.google.com/sickle_cell_image

G. Precautions

Gloves and

laboratory coat were constantly worn to prevent contact with blood samples. Apparatus (i.e. the tiles pipette and can tubes) used were well washed and dried to prevent contamination of the results each time it was used. Rocking of samples on tile was done gently to prevent flowing of samples into each other. Reagents not in used were stored as directed by the manufacturer and allowed to reach room temperature prior to use.

Determination of Gene (Allele) Frequency Using The Hardy-Weinberg Law WHEN TWO ALLELES ARE INVOLVED

When two alleles are involved in the determination of the trait of a particular phenotype in which case gene exist as just two alleles, for instance as in the of Rhesus (D) antigen then the expression for genotype frequency is the binomial expansion of

$$P + q = 1$$
, i.e.
 $P^2 + 2pq + q^2 = 1$

Let **D** represent the dominant allele for the Rhesus D antigen

d represent recessive allele Therefore their genotype follows as
DD= homozygous positive (Rhesus positive)
Dd = heterozygous positive (Rhesus positive)
Dd = homozygous negative (Rhesus negative)

For example the primary data's gene frequencies for the Rhesus antigen can be worked as for as follows Let \mathbf{p} = the frequency of \mathbf{D} allele and \mathbf{q} = the frequency of \mathbf{d} allele.

$$q^2 = 0.213$$

Therefore $q = \sqrt{0.213}$
 $q = 0.462$
But $p + q = 1$, $p = 1 - 0.462$
 $P = 0.538$

The gene frequency of the D = 0.538 and d = 0.462

It follows that their genotypic frequencies will be calculated as

 $DD = 0.538 \times 0.538 = 0.289$ $Dd = 2 \times 0.538 \times 0.462 = 0.497$ $dd = 0.462 \times 0.462 = 0.213$

WHEN THREE ALLELES ARE INVOLVED

In the case where the gene involves three alleles then additional term is added to the equation P + q = 1 so as to get multinomial expansion i.e. P + q + r = 1, taking the additional term to be '**r**' This can be used to calculate the allele frequencies of the ABO blood groups, it then follows that their genotypic frequencies would follow $p^2 + 2pr + q^2 + 2qr + 2pq + r^2 = 1$

Let p = the frequency of A allele q = the frequency of the B allele r = the frequency of the O allele

Using the secondary as an example to calculate the allele (gene) and genotypic frequencies; Using the binomial expansion of the expression below the allele frequencies for the ABO blood groups can be calculated.

$$(P + r)^{2} = \{f (A) + f (O)\}^{2}$$

= f (AA) + 2 f (AO) + f (OO)
P (A) = $\sqrt{f} \{(A-type) + (O-type)\} - r$
But r² = 0.513
r = $\sqrt{0.513}$
= 0.716
Allele O is 0.716
P (A) = $\sqrt{\{(0.227) + (0.513)\}} - 0.716$
= 0.861 - 0.716
= 0.145
Allele A is 0.145
Therefore using p + q + r = 1
0.145 + q + 0.716 = 1
q = 1 - 0.861
= 0.139
Allele B is 0.139
Therefore their respective frequencies are
AA = 0.145 x 0.145 = 0.021
AO = 2 x 0.145 x 0.716 = 0.208
BB = 0.139 x 0.716 = 0.208
BB = 0.139 x 0.716 = 0.199
AB = 2 x 0.145 x 0.716 = 0.199
AB = 2 x 0.145 x 0.716 = 0.513

IV. DISCUSSION

The study determined the distribution of the ABO/Rhesus blood group phenotypes and genotypes as well as sickle cell trait phenotypes among patients who visited the KNUST hospital laboratory within a period of 5 months. Blood group O was found to be the commonest, occurring in more than half of the people studied. Blood groups A and B which occurred in approximately equal proportions were also more prevalent than blood group AB, which was the least abundant. Comparing the primary and secondary data revealed a correlation between both data sets which were not significantly different (P = 0.135) and therefore a confirmation of the frequencies of occurrence of the blood groups. Thus while in the secondary data the frequencies were 51.3%, 22.7%, 22.3% and 3.7% for O, A, B and AB respectively, in the primary data the proportions were also 52.8%, 21.0%, 20.9% and 5.3% for O, A, B and AB respectively. This finding is in accord with data in many countries of the world (http://www.bloodbook.com/world-abo.html). A case in point is the finding in many parts of the world where the prevalence of blood group O was 42.6%, A was 39.1% which was higher than the 13.6% of B while AB had the least proportion of 4.9%. The similarity in frequencies of the various blood groups in both data sets which is in accord with literature confirms their stability.

The current work revealed that blood group O has the highest frequency of occurrence among the various ethnic groups in Ghana with the exception of the Guans. This is in agreement with the report from earlier studies of the ABO blood groups system among Ghanaians and other West Africans which have given the prevalence of the blood group O as 50%, AB about 3% and groups A and B in about equal proportions with B being slightly more common than A in the majority of tribes (Worlledge *et al.*, 1974; MacGeoch *et al.*, 1992; Acquaye, 2001 and Dacic *et al.*, 1981). According to Acquaye (2004), blood group O has the highest frequency among the Akans, ranging from 50% to 57%. This is similar to our results of 56% for the blood group O among the Akans. Again, our findings of 19.9% of A for the Akans agree with Acquaye's data for blood groups A which ranged between 19.1% and 22.6%. With blood group AB, though the current data of 5.4% differ numerically from Acquaye's result of 1.3% to 2.5%, the fact remains that

both data sets are in agreement that among the Akans, blood group AB has the least frequency of distribution. These differences between the ABO blood groups among the ethnic groups were found to be significant (P = 0.005).

The frequency of the rhesus trait was 78.7% positive with only 21.3% negative in the primary data which again corroborated the frequency of 92.5% positive and 7.5% negative in the secondary data. Thus results of one data set were not significantly different from the other (P = 0.594). This also is in agreement with data from some parts of the world (http://wikipedia.org/wiki/Blood type).

The frequency of occurrence of the sickle cell trait in the primary and secondary data was 81.6% and 88.7% negative respectively and with 18.4% and 11.3% being positive respectively, both data sets providing insignificantly different results (P = 0.423). The distribution of the sickle cell traits among the ethnic groups showed a similar pattern as in the primary and secondary data.

The results agree with the assertion by Ohene-Frempong (2001) that one out of fifty Ghanaian children is born with the sickle cell disease. Comparing this startling results with the report of one out of 500 African- America Children born with the disease in the United States (Mary Kugler, 2009), one must admit that Ghanaians are not much aware of how the sickle cell disease can be avoided through genetic counseling before marriage.

A. Conclusion

The highest occurring frequency of the ABO blood group system in the of study area and therefore, in Ghana is group O, with the least being AB. Both groups A and B occur in about equal proportions. Likewise, both Rhesus and sickle cell traits are highly common in Ghana.

B. Recommendation

There is therefore the need for premarital genetic counseling in order to reduce the phenomena of erythroblastosis fetalis and sickle cell disease in the country. It is also recommended that adequate preparation is made for vaccination of rhesus negative women who are giving birth to rhesus positive children for the first time to reduce the frequency of the hemolytic disease of the new born. More work should be done on the ABO/Rhesus blood groups and sickle cell trait in Ghana to establish the frequency of distribution of the various blood groups in the country as well as among the various ethnic groups.

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