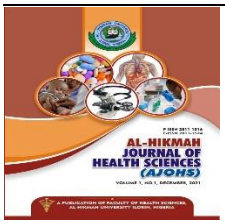
P-ISSN: 2811-1516
E-ISSN: 2811-1524



AL-HIKMAH JOURNAL OF HEALTH SCIENCES (AJOHS)
Official Publication of Faculty of Health Sciences,
Al-Hikmah University Ilorin, Nigeria
ww.alhikmahuniversity.edu.ng/centralJournal

Original Article

A Correlate of Awareness and Prevalence of Sickle Cell Disorder Phenotypes and Carrier Status of Undergraduate Students of Achievers University Owo

Toyeeb O. Mohammed, Ramat. O. Mohammed Nasr, AbdulWasii Hassan

[1]Department of Medical Laboratory Sciences Al-Hikmah University Ilorin, Kwara State, Nigeria 240212
[2]Department of Obstetrics and Gynecology University of Ilorin Teaching Hospital Ilorin, Kwara State, Nigeria 240001
[3]Department of Medical Laboratory Science, Achievers University Owo, Ondo State, Nigeria

ARTICLE INFO

Article history:
Received 29 May 2021
Accepted 10 September 2021
Available online 30 October 2022

Keywords
Sickle cell disease,
Haemoglobin,
Ethylene Diamine Tetra-acetic Acid,
HbSS,
HbAS,
HbAC,
HbSC

Corresponding Author*:*
Mohammed Toyeeb O
Department of Medical Laboratory Science, Faculty of Health Sciences, Al-Hikmah University Ilorin, Kwara State, Nigeria.
Phone Number: +2347036284843
Email:toyeebmohammed8@gmail.com/ toyeebee@gmail.com
Zip code: 240212

ABSTRACT

Sickle cell disease (SCD) is the most common single-gene blood disorder in the world, representing a significant public health problem. More than a thousand variants of haemoglobin exist but fortunately, only a few are considered significant clinically. The study was a cross sectional prevalence study aimed at evaluating the level of awareness and prevalence of sickle cell disorder among undergraduate students of Achievers University, Owo. A simple randomly selected 100 students made up of both gender who consented to the study were recruited. Blood samples were collected from the participants into Ethylene Diamine Tetra Acetic Acid (EDTA) and analysed by cellulose acetate membrane electrophoresis method. The results of the study show a prevalence of 8% sickle cell disease and 30% carrier. More females (66) participated in the study than males (34) and had a significantly higher prevalence of HbSS, HbAC and HbSC than the male's counterpart ($p > 0.05$). However, males had more HbAS than the females. Muslim participants had more sickle cell disorder than the Christians. In the same vain, participants from Yorubas. Yorubas had more HbAS, and HbSC while the Igbo had more HbSS and HbAC. The association between religion or ethnicity and prevalence of SCD was statistically not significant ($p < 0.05$). In conclusion, understanding knowledge about sickle cell inheritance, its health and reproductive health implications as well as behaviour towards individual with SCD particularly is important regarding limiting the spread of the diseases. The study recommends continuous enlighten on the possibility of eradicating this disorder and incorporation of genetic counselling in premarital counselling.

Please cite this article as: Mohammed, T.O., Mohammed Nasr R.O. & Hassan, A. (2021). A Correlate of Awareness and Prevalence of Sickle Cell Disorder Phenotypes and Carrier Status of Undergraduate Students of Achievers University Owo, *Al-Hikmah Journal of Health Sciences, 1*(1), 43-48.

Introduction

The Haemoglobin molecule is the protein pigment responsible for transporting oxygen from the lungs to the tissues for energy. The molecule consists of the iron containing heme unit and a globin portion which comprises four polypeptide chains. The production of the globin moiety is governed by specific genes whose mutations result in the production of haemoglobin variants which differ in structure and function from the normal haemoglobin (A) molecule (Thom *et al.*,

P-ISSN: 2811-1516
E-ISSN: 2811-1524

2013). Haemoglobin variants are characterized either as quantitative, when there is an impairment in the production of globin proteins (Thalassemias) or as qualitative resulting in the production of structurally abnormal globin protein (Thom *et al*., 2013). More than a thousand variants of haemoglobin exist but fortunately only a few are considered significant clinically (Giardine *et al*., 2011) These include Hb S, C, D, E and the thalassemias with the most widespread variants being S, C and E (Weatherall *et al*., 2006). In Africa, Hb S is the most common and clinically significant haemoglobin variant. Although, individuals who are carriers of these haemoglobin variants may be asymptomatic or have mild anaemia, inheritance of two copies of the same variant or two abnormal variants may result in severe forms of haemolytic disease.

Sickle cell disease (SCD), the most common single-gene blood disorder (hemoglobinopathy) in the world, represents a significant public health problem in India. In India, SCD is highly prevalent in the Western, Central, and Eastern regions and in pockets of the South in the states of Maharashtra, Madhya Pradesh, Orissa, Andhra Pradesh, Gujarat, Chattisgarh, Tamil Nadu, and Kerala (Chandrashekar and Soni, 2011). Sickle cell disorders (SCD) are autosomal recessive disorders in which HbS is inherited alongside another abnormal Hb variant (Adekile and Adeodu, 2007).

SCD is an inherited disorder of the red blood characterized by vaso-occlusive pain crises, risk for pneumococcal infections, acute chest syndrome, stroke and organ failure and is associated with substantial morbidity and premature mortality (Balgir, 2007). Sickle cell disease limits the oxygenating role of hemoglobin, resulting in the damaging or the "sickling" of the red blood cells (Barakat *et al*., 2008). This disorder affects all parts of the human body and differs widely among individuals (Bloom, 2005). Sickle cell disorders have remarkable public health implications for Africa. Most of the affected children born in high income countries survive with the chronic disorder whereas a significant proportion born in low income countries die before the age of five years with appreciable proportion reportedly undiagnosed. They contribute the equivalent of 3.4% to under five deaths worldwide with up to 6.4% in Africa (Modell and Darlison, 2008). 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" (Ogamdi, 1994). The sickleshaped red blood cells described by Herrick caused several complications, including chronic anemia, vaso-occlusive pain episodes, ischemic organ damage, infections, small stature, and delayed puberty (Barakat et al., 2008). 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 (Reid & Rodgers, 2007). Nigeria has the largest burden of sickle cell disorders in the world with over 40 million people being carriers of the S gene and about 150,000 children born each year with sickle cell anaemia (Williams and Weatherall, 2012).

Although HbSS is the most common form of sickle cell disorder in Nigeria, HbSC and Sβ thalassemia are also seen (The Federal Ministry of Health, 2014). Early detection of these variants via newborn screening is an important public health approach to identify affected children before they develop complications. It allows for early enrolment in comprehensive specialty care programmes, early institution of prophylactic therapies and parental education to recognise serious complications (Yusuf *et al*., 2011). 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 (Cerami, 1974). Also, the life expectancy for SCD has doubled since the 1960s. Before that time, few patients lived to reach adulthood (Platt *et al*., 2014).

Methodology

The research was carried out among Achievers University students in Owo, Ondo State, Nigeria. Owo is situated in south-western Nigeria, at the southern edge of the Yoruba Hills, and at the intersection of roads from Akure, Kabba, Benin City, and Siluko. Owo is situated halfway between the towns of Ile Ife and Benin City. Achievers University Owo, is a private sector initiative, established in 2007 and accredited by the National Universities Commission. It is located on land in the Idasen community of Owo, consisting of Ulale 1, Ulale 11, Ulema, Ijegunma, Isijogun and Amurin Elegba. The university sprang from the Achievers Group of Education and Training Organisation, located in Ibadan Oyo State of Nigeria owned and run by Hon, Bode Ayorinde and other educationalist. The university commenced academic activities during the 2007/2008 academic session.

P-ISSN: 2811-1516
E-ISSN: 2811-1524

Study Population
A total of one hundred (100) subjects was enrolled in this study which was comprised of undergraduate students of Achievers University Owo in Ondo state, Nigeria.

Ethical Approval
The approval of the Ethical Review Committee of the department of Medical Laboratory Science, Achievers University Owo, was be obtained. All experiment were performed in accordance with Good Laboratory Practice (GLP) regulations.

Collection and Processing of Blood Specimen
Four and a half milliliters (4.5mls) venous blood was collected under aseptic conditions and with minimal stasis from each subject using sterile syringe and needle from the ante-cubital vein. The blood sample will then be added into a commercially prepared Ethylene Diamine Tetra Acetic Acid (EDTA) plastic tube. Blood samples were processed within four hours of blood collection. Hemoglobin electrophoresis was performed using Cellulose Acetate Electrophoresis at alkaline pH Method (Shrestha and Karki, 2013).

Hemoglobin Electrophoresis
The cellulose acetate membrane Hemoglobin electrophoresis method at pH 9.2 adapted after (Junius and Martin, 1991), was used to confirm the results generated by the sickling test methods.

Principle

This method is based on the fact that proteins normally have either positive or negative charge that is determined by the charged amino acid they contain. When an electric field is applied to a solution containing protein molecules, positively charged proteins will migrate to the cathode and negatively charged protein will migrate to the anode. Depending on their charges, size and shapes, different haemoglobin will separate and migrate at different rates. They are then stained and their band compared with known control (Okwi *et al*., 2010).

Procedure

1. Less than 5µL of blood will be mixed with deionized (DI) water to lyse the RBC and release Hb content into the solution.
2. Less than 1µL of the solution will then be stamped onto the cellulose acetate paper.
3. An electric field of about 150-200volts will be applied, that causes the hemoglobin types to travel different distances across the paper strip.
4. A control containing known abnormal hemoglobins will be run along with the unknown patient samples.
5. The cellulose acetate gel will be stained with ponceau red.
6. The band formed will compared with that of known band.

Statistical Analysis
The result obtained will be organized and subjected to appropriate statistical analysis. Statistical Package for Social Science (SPSS) version 21 will be used in all the statistical analysis. Data from 2 groups will be compared using students 2 tailed t-test for paired samples. Data between groups was also be compared using a one-way analysis of variance (ANOVA). The level of significance is fixed at $P<0.05$.

Results
Results would be presented in tables, figures and expressed as mean ±standard deviation. Pearson's chi-square and inferential statistical analysis of data generated from the 100 undergraduate students who consented to the study is presented in the table below. The average age of the participants was 22.54 ± 3.41, made up of students of both genders from different Departments of the University and at different levels of study.

Table 1 shows the sex and age-related prevalence of sickle cell disorder and carrier status among the undergraduate students. A total of 8 (8.0%) had sickle cell disorder while 30 (30%) were carriers. Females that participated in the study were 66 out of which 44 (66.7%) were HbAA, 12 (18.2%) were HbAS, 2 (3.0%) were SS, 6 (9.1%) were HbAC and 2 (3.0%) were HbSC while the male participants were 34, made up of 18 (52.9%), 12 (35.3%), 4 (11.8), 0 (0%) and 0(0%) HbAA, HbAS, HbSS, HbAC and HbSC respectively. The Pearson's chi-square analysis show a strong association between sex and sickle cell disorder and carrier status ($p = 0.034$). Participants within the ages of 21 and 25 years made up the majority of the sample population with 36 (60.0%) HbAA, 14 (23.3%) HbAS, 4 (6.7%) HbSS, 4 (6.7%) HbAC and 2 (3.3%) HbSC while those ≤ 20 years were 24 made up of 14 (583%), 8 (33.3%), 0 (0%), 2 (8.3%) and 0 (0%) HbAA, HbAS, HbSS, HbAC and HbSC respectively and those ≥ 26 years were 16, made up of 12 (75.0%), 2 (16.7%), 2 (16.7%), 0 (0%) and 0 (0%) HbAA, HbAS, HbSS, HbAC and HbSC respectively. The association between age and prevalence of sickle cell disorder and carrier status was not statistically significant ($p = 0.488$)

Table 2, shows the participants awareness and prevalence of sickle cell disorder and carrier status.

P-ISSN: 2811-1516
E-ISSN: 2811-1524

Those who had a good knowledge of genotype were 94, made up 660 (63.8%), 22 (23.4%), 6 (6.4%), 4 (4.3%) and 2 (2.1%) HbAA, HbAS, HbSS, HbAC and HbSC respectively. A strong association was observed between knowledge of genotype and prevalence of sickle cell disorder and carrier status (p = 0.049). Out of the 100, 92 knew their genotype prior to the study out of which 58 (63.1%) were HbAA, 20 (21.7%) were HbAS, 6 (6.5%) were HbSS, 6 (6.4%) were HbAC and 2 (2.2%) were HbSC.

Table 1: Sex and age-related prevalence of sickle cell disorder and carrier status

Variables	Number of examine	Number of HbAA (%)	Number of HbAS (%)	Number of HbSS (%)	Number of HbAC (%)	Number of HbSC (%)	p-value
Sex							
Male	34	18 (52.9)	12 (35.3)	4 (11.8)	0 (0)	0 (0)	0.034
Female	66	44 (66.7)	12 (18.2)	2 (3.0)	6 (9.1)	2 (3.0)	
Total	100	62 (62.0)	24 (24.0)	6 (6.0)	6 (6.0)	2(2.0)	
Age group (years)							
≤ 20	24	14 (58.3)	8 (33.3)	0 (0)	2 (8.3)	0 (0)	0.488
21 – 25	60	36 (60.0)	14 (23.3)	4 (6.7)	4 (6.7)	2 (3.3)	
≥ 26	16	12 (75.0)	2 (16.7)	2 (16.7)	0 (0)	0 (0)	

Table 2: Participants awareness and prevalence of sickle cell disorder and carrier status

Variables	Number of examine	Number of AA (%)	Number of AS (%)	Number of SS (%)	Number of AC (%)	Number of SC (%)	p-value
Knowledge of sickle cell disorder							
Yes	94	60 (63.8)	22 (23.4)	6 (6.4)	4 (4.3)	2 (2.1)	0.049
No	6	2 (33.3)	2 (33.3)	0 (0)	2 (33.3)	0 (0)	
Know your genotype							
Yes	92	58 (63.1)	20 (21.7)	6 (6.5)	6 (6.5)	2 (2.2)	0.424
No	8	4 (50.0)	4 (50.0)	0 (0)	0 (0)	0 (0)	

Discussion

Sickle cell disorder has been viewed as a global problem of public health concern which has occasioned the establishment of sickle cell centers in each of the six geopolitical zones in Nigeria. The present study evaluated the level of awareness and prevalence of sickle cell disorder and carrier status which facilitate and contribute to optimum utilization of the centers.

Based on the findings of the study, a strong association was observed between sex and prevalence of sickle cell disorder and carrier status (p >0.05), this could be due to higher female participation in the study than the males. The study report a prevalence of sickle cell disorder of 8% and a prevalence of 30% carriers. HbAA genotype had the highest prevalence (62.0%), followed by HbAS (24.0%), HbSS and HbAC (6.0%) and HbSC (2.0%). Females were more of HbAA than males while the males were more of HbAS and HbSS than the females. Like in the findings of earlier study (Akhigbe *et al*., 2009), no incidence of HbAC and HbSC was observed among the males but contrary to the result of Pennap *et al*. (2011) that suggested that gender have no effect on the incidence of haemoglobin variants. Our findings corroborate the report of Martinez et al. (2014), who reported a HbS carrier prevalence of 25-30% and similar to the findings of Akhigbe *et al*. (2009) in Ogbomoso. In their study among the students of LAUTECH, the prevalence of HbAA, HbAS, HbAC, HbSS and HbSC were 71.03%, 22.19%, 5.2%, 0.54%, 0.80% and 0.18% respectively. Our findings is also in consonance with the study of Bakare et al. (2004) that reported a prevalence of 3.0% and 2.0% for HbSS and HbSC respectively, the study of Nwafor and Bamigo (2001) that reported a prevalence of 4% and 1% for HbSS and HbAC respectively and Osaro (2010) in the Niger Delta of

P-ISSN: 2811-1516
E-ISSN: 2811-1524

Nig[illegible] nals, internet, friends and 29.4[illegible] sickle cell anemia (HbS).

The findings of this study will straighten the records on the haemoglobin variants associated disease conditions. HbAA has been reported to be more susceptible to plasmodial parasite infection than HbAS and HbSS, more vulnerable to malaria than other haemoglobin variants (Uzoegwu and Onwurah, 2003). However, the findings of this study was lower than the findings of Awaitey et al. (2020) that reports a prevalence 16.7% made up of 6.2% and 7.9% of HbSS and HbSC respectively and the 22% HbAS, 11% HbAC and 22% HbSS among blood donors in Illorin (Durotoye et al., 2021). The low prevalence of HbSS and HbSC observed in this study could be occasioned by differences in awareness of sickle cell disorder, increased awareness on the importance of genotyping prior to marriage and childbearing and improved socio-economic conditions. Based on this findings, there is possibility of having a lower or 0% population of sickle cell disorder in near future as inheritance of one copy of an abnormal haemoglobin variant (C and S) in addition to an S haemoglobin makes an individual diseased (Ashish et al., 2008). A non statistically significant association was observed between haemoglobin variants and age. This is as expected as age has no effect on haemoglobin after six months old (Malcorra, 2001).

The majority of the participants were aware of their genotype and out of the 94% were aware of sickle cell disorders as an inherited genetic disorder and its effect on the red blood cell. The prevalence of haemoglobin variants and the knowledge of sickle cell disorder was observed to be statistically significant ($p < 0.05$). The findings of this study is in support of the previous studies of undergraduate medical students in Lagos and secondary school students in Jos were 84% and 83% of the respondents claimed to have heard about sickle cell disorder (Animasahun and Akitoye, 2009; Olakunle et al., 2013) and disagree with the earlier studies among adolescents in India and high school students in Jamaica were 49% and 46.2% respectively affirmed the genetic transmission of the disorder (Desai and Serjeant, 1976; Vasava et al., 2009).

In the study area, sickle cell disease as well as other related conditions are incorporated in secondary school teaching curriculum and taught as part of basic science in secondary school, culminating in increased awareness. In addition, with increasing dependence on social media for information, level of awareness on sickle cell disease could be widened. Other sources of information claimed by the participants and in conformity with the report of Dasai and Serjeant

The high level of awareness and knowledge of sickle cell disorder may not be surprising since the participants were mainly university students, who were likely to be healthy and possessed normal hemoglobin in order to withstand the usual academic stress, as opposed to their sickle cell counterparts, who hardly survived before they could gain admission into a higher institution.

Sickle cell disorder is a preventable disease with a simple, cost-effective interventions such as early detection of sickle cell disease individuals by screening of newborn otherwise referred to as newborn screening (NBS). The newborn screening program allows for early diagnosis, parental education, and comprehensive care, which results in a marked positive impact on mortality and morbidity throughout infancy, childhood, and adulthood in many countries where it is being practiced. NBS can also help to assuage the previous observation that children with sickle cell disease have a high risk of dying from associated complications without being diagnosed at all due to the lack of universal newborn screening in most low-income countries (Ndubila, 2013).

Summary and Conclusion

The study report shows a 8% and 30% sickle cell disease and carrier respectively. Though high, but within the average range as published by WHO, were lower than the report of previous studies. Indicating the possibility of reduced or minimal future prevalence if more awareness as well NBS is encouraged. Knowledge in hemoglobin variants and carrier status is thus necessary in reproductive decision making to modify risk for offspring of serious conditions such as sickle cell anemia as well as provide direct health benefit to carriers. If sickle cell disease control strategies must yield any significant results, there is a need to raise awareness about SCD, especially among adolescents in secondary and tertiary institutions in Nigeria is recommended. Therefore, understanding knowledge about sickle cell inheritance, its health and reproductive health implications as well as behaviour towards individual with SCD particularly is important regarding limiting the spread of the diseases.

Acknowledgement

I acknowledge the effort of Dr (Mrs) Mohammed-Nasir Rahmat in getting some of the materials used for this work.

References

Adekile, A.D., Adeodu, O.O. (2007). Haemoglobinopathies. In: Textbook of

P-ISSN: 2811-1516
E-ISSN: 2811-1524

Paediatrics and Child health in a tropical region. *African educational services*; 2:22-26.

Akhigbe, R. E., Ige, S. F., Afolabi, A. O., Azeez, O. M., Adegunlola, G. J. and Bamidele, J. O. (2009). Prevalence of Haemoglobin variants, ABO and Rhesus blood groups in Ladoke Akinlola University of Technology, Ogbomoso, Nigeria. *Trends in Medical Research*, 4(2):24-29.

Animasahun, B. A. and Akitoye, C. O. (2009). OF Sickle cell anaemia: awareness among health professionals and medical students at the Lagos University Teaching Hospital, Lagos. *Nig Q J Hosp Med*. 19(4):195-199

Araujo, A.N. (2009). Acute splenic sequestration in children with sickle cell anemia. *Journal de Pediatria*; 85:373–374.

Ashish, K., Raseswari, P. and Pruthviraj, S. (2008). Perinatal outcome in pregnancy with sickle cell anemia. *The Journal of Obstetrics and Gynecology of India*, 58:500–503.

Bakare, A. A., Azeez, M. A. and Agbolade, J. O. (2004). Gene frequencies of ABO and Rhesus blood groups and hemoglobin variants in Ogbomoso, South-West, Nigeria. *Global J. Med*. Sci. 3:17-22

Balgir, R. (2007). Epidemiology, Population Health Genetics and Phenotypic Diversity of Sickle Cell Disease in India. *The Internet Journal of Biological Anthropology*; 1(2)92-95.

Barakat, P., Simon, K., Schwartz, L., Radcliffe, J. (2008). Correlates of pain-rating concordance for adolescents with sickle cell disease and their caregivers. *Clinical Pain Journal*; 24(5):438-446.

Bhatnagar, P., Purvis, S., Barron-Casella, E., DeBaun, M.R., Casella, J.F., Arking, D.E., Keefer, J.R. (2011). Genome-wide association study identifies genetic variants influencing F-cell levels in sickle-cell patients. *European Journal of Human Genetics*; 56:316.

Bloom, M. (2005). Understanding sickle cell disease. Jackson, MS: *University Press of Mississippi*.

Brousse, V., Buffet, P., Rees, D. (2014). The spleen and sickle cell disease: The sick (led) spleen. *British Journal of Haematology*; 166:165–176.

Cerami, A. (1974). Sickle cell anemia. *New York: Joseph Okpaku Publishing Co. Inc*.

Chakravorty, S., Williams, T.N. (2015). Sickle cell disease: A neglected chronic disease of increasing global health importance. *Archive of Disease in Children*; 100:48–53.

Chandrashekar, V., Soni, M. (2011). Hemoglobin disorders in South India. *International Scholarly Research Network in Hematology;* 11:748939.

Colombatti, R., Martella, M., Cattaneo, L., Viola, G., Cappellari, A., Bergamo, C., Azzena, S., Schiavon, S., Baraldi, E., Dalla Barba, B. (2019). Results of a multicenter universal newborn screening program for sickle cell disease in Italy: A call to action. *Pediatric and Blood Cancer*; 66: e27657.

Costanzo, V.L. (2011). Sickle Cell Trait Counseling for Student Athletes. *Human Genetics*; 4:89-93.

De-Montalembert, M. (2012). Management of children with sickle cell anemia: A collaborative work. *Archive of Pediatrics*; 9:1195–1201.

Desai, P., and Serjeant, G. R. (1976). Awareness of Sickle Cell Disease among High School Students in Kingston, Jamaica. *Biokemistri*, 91(3): 265-7

Durotoye, I. A., Salaudeen, A. G., Sanni, E. O., Babatunde, A. S., Durowade, A. K., Olawumi, H. O., Akande, T. M. and Musa, O. I. (2021). Determination of normal and variant hemoglobin using capillary electrophoresis among voluntary blood donors in north central Nigeria: Implication on blood transfusion services. *Sudan Journal of Medical Sciences*, 15(1):, DOI 10.18502/sjms.v16i1.8935

Eastman, J.W., Wong, R., Lao, C.L., Morales, D.R. (1996). Automated HPLC screening of newborns for sickle cell anemia and other hemoglobinopathies. *Clinical Chemistry*; 425:704–710.

Ferrone, F., Nagel, R.L. (2010). Sickle hemoglobin polymerization, in Disorders of hemoglobin: *Genetics, pathophysiology, clinical management. Cambridge University Press*; 2:19-25.

Frenette, P.S., Atweh, G.F. (2007). Sickle cell disease: Old discoveries, new concepts, and future promise. *Journal of Clinical Investigations*; 117:850–858.