THE PREVALENCE OF SICKLE CELL TRAIT AMONG ADOLESCENTS IN BUNGOMA COUNTY, KENYA.

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RESEARCH THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT OF THE DEGREE OF MASTER OF MEDICINE IN CHILD HEALTH AND PAEDIATRICS OF MOI UNIVERSITY.

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DECLARATION:

Student's declaration:

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DEDICATION

I dedicate this work to my father, the late Mr. Stephen Watenga Chemengu for encouraging me to work on my dreams and make the world a better place.

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ABBREVIATIONS, ACRONYMS.

Hb	Haemoglobin.
HbAA	Normal haemoglobin.
HbAC	Carrier state of Haemoglobin C disease.
HbAS	Carrier state of sickle cell disease also known as the sickle cell trait.
HbCC	Haemoglobin C disease.
HBE	Hb Electrophoresis.
HbF	Foetal Haemoglobin.
HbSC	Milder form of haemoglobinopathy compared to sickle cell with both haemoglobin C and S.
HbSS	Abnormal variant of haemoblobin responsible for sickle cell disease.
HPLC	High performance liquid chromatography.
IEF	Isoelectric Focusing.
SCD	Sickle Cell disease.
SCT	Sickle cell trait.
WHO	The World Health Organization.

DEFINITIONS.

Adolescent- Any person aged between 10 and 19 years.

Sickle cell trait- Carrier state of sickle cell disease, where there is one normal gene and one sickle cell gene. Also documented as HbAS.

Haemoglobin variants- refers to HbAA,HbAS,HbAC,HbSS,HbSC,HbCC.

Sickle cell disease- refers to HbSS, HbSC variants.

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THE PREVALENCE OF SICKLE CELL TRAIT AMONG ADOLESCENTS IN BUNGOMA COUNTY, KENYA

ABSTRACT

Background: Sickle cell disease (SCD) is a genetic disorder inherited in autosomal recessive fashion and affects the haemoglobin (Hb) molecule. The homozygous state (HbSS) is the severest form of SCD and the heterozygous state(HbAS) is the Sickle cell trait or carrier state (SCT). Health education, genetic counseling, testing and identifying persons with the sickle cell gene and advising them on how to prevent birth of children with SCD are important primary intervention strategies for control of SCD.

Objectives: To determine the prevalence of sickle cell trait among adolescents attending mixed day secondary schools in Bungoma County. To determine the haemoglobin variants among adolescents attending mixed day secondary schools in Bungoma County. To test the association between the demographic characteristics and sickle cell trait among adolescents attending mixed day secondary schools in Bungoma County.

Methods cross-sectional study was done on adolescents attending mixed day secondary schools in Bungoma County of Western Kenya between January 2017 and July 2017. Stratified random sampling was applied; the county was stratified into 9 constituencies; a mixed day secondary school was selected randomly from each constituency. Public health education on SCD control was offered to 2389 students in 9 schools, 2248 met eligibility criteria. A total of 225 eligible participants were selected randomly from the school registers and consented. Pretest counseling was done followed by testing for sickle cell gene status using a point of care (POC) device (The Sickle scan™). Post test counseling was offered. Demographics and test results were documented. Proportions and Percentages was used to describe categorical data; Mean and Median for continuous data. Logistic regression was done and risk ratios (RR) generated, Pearson chi square was used to test for associations. Level of significance was set at p-value <0.05 with Confidence interval at 95%.

Results: All 225 participants consented for testing; Males: 116/225(51.6%), Females: 109/225(48.4%). The median age was 17 years. HbAS prevalence was 18.7% (42/225) (CI 0.14-0.24). Proportion of males with HbAS was 24/116(20.7%); proportion of females with HbAS was 18/109(16.5%). Proportion of HbAS among students with known family history of SCD was 6/12 (50%);proportion of HbAS among those with no known family history of SCD was 36/213 (16.9%).Of those with family history of SCD; 1/12(8.3%)reported a sibling,9/12(75%) reported a cousin and 2/12(16.6%)reported other relatives. Hb variants were HbAA (183/225,81.3%) and HbAS (42/225,18.7%), there were no other Hb variants. There was no invalid result. Those with Family History of SCD were three times more likely to have HbAS(RR-2.958, p-value= 0.004,CI:1.561-5.607).

Conclusion: Prevalence of HbAS among adolescents is high. Hb variants found were HbAA and HbAS, there was no HbSS or HbC variants found. Having a Known Family History of SCD was significantly associated with having HbAS.

Recommendations: Public Health Education on SCD and SCT to Bungoma County residents. Setting up of genetic counseling and testing centers for SCD and SCT in Bungoma County.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Sickle cell disease (SCD) was first described by James B. Herrick in 1910. It is a genetic disorder that affects the haemoglobin molecule.

The haemoglobin molecule is constituted by four globin chains; Genes in the α-globin and β-globin clusters on chromosome 16 and 11respectively control the production of globin chains(Iyer, Sakhare, Sengupta, &Velumani, 2015). Point mutation result in haemoglobin gene variants which fall into two broad groups – structured variants in which there is a change in the sequence of amino acid producing unusual haemoglobin (haemoglobinopathy) and thalassaemia in which there is a lower or abolished production of globin chains. Sickle cell anaemia is caused by a point mutation in the β-globin chain of haemoglobin causing the hydrophilic amino acid glutamate to be replaced by the hydrophobic amino acid valine at the sixth position (Malowany& Butany, 2012). Fetal haemoglobin (HbF) has two alpha and two gamma chains (a_2y_2) and Adult haemoglobin (HbA) has two alpha and two beta chains ($a_2\beta_2$)(Domínguez, Zurita, Calvopiña, Villacís, & Mora, 2013).

SCD is inherited in an autosomal recessive fashion. A person who has two normal genes has normal haemoglobin (HbAA)(Malowany J.I., 2012). A person who receives a gene for sickle cell disease from one parent and a normal gene from the other has a condition called sickle cell trait (heterozygous state HbAS). Two genes of the sickle hemoglobin, each one inherited from both parents results in the disease(homozygous state HbSS). If both parents are heterozygous for the HbS mutation (HbAS), there is a 25% chance per pregnancy of having an offspring with Sickle cell disease(HbSS) and 50% chance of the offspring being a carrier (HbAS) and a 25% chance of the

offspring having the normal Haemoglobin (HbAA). If one parent is homozygous (HbSS) and the other parent is heterozygous (HbAS), there is a 50% chance for every pregnancy of having an offspring with Sickle cell disease (HbSS) and a 50 % chance of having a carrier (HbAS). If one parent is homozygous (HbSS) and the other is normal (HbAA), then every pregnancy is likely to result into an offspring who is a carrier (HbAS). If both parents are homozygous (HbSS) then every pregnancy is likely to result into an offspring with sickle cell disease (HbSS). It is advisable that persons who have the gene for sickle cell (HbAS or HbSS) should have offsprings with persons with normal genes(HbAA) in order to avoid the likelihood of getting offsprings with HbSS.

Other compound heterozygous states include sickle-haemoglobin C (HbSC), sickle beta-zero thalasaemia (HbSβ°), sickle beta-plusthalasaemia (HbSβ^{+}), sickle-haemoglobin D-Punjab (HbSD-) among others. (Malowany J.I., 2012)

Sickle cell trait is the carrier state of the disease. Individuals with sickle cell trait are mostly healthy, live a normal life (John, 2010) but contribute towards transmission of the sickle cell gene. They have potential of giving birth to children with sickle cell disease.

Persons with homozygous state of the disease(HbSS) and compound heterozygous states such as sickle-haemoglobin C (HbSC) are symptomatic. Symptoms usually begin after six months of life relating to decline of fetal haemoglobin levels. They present with signs and symptoms of anaemia, infection and vaso-occlusive crisis such as jaundice, pallor, fever and severe pain. (Lazarus, 2011)

Sickle cell disease poses a significant public health burden in sub-Saharan Africa, and especially in the malaria endemic regions where the gene frequency is highest. In

these regions, it is a significant cause of morbidity and mortality and control programs become essential. Despite the need for control programs, most nations in the affected areas do not have control programs.(WHO, 2006.)

In Kenya, the sickle cell gene is found mostly in the lake regions of western Kenya and the Coastal areas, these are malaria endemic regions.(Aluoch JR, 1993)

In terms of control strategies, it is paramount to note that at the 59th World Health Assembly, having recognized the public health burden associated with sickle cell disease, recommendations were proposed for control of the disease. First, that national programs for prevention and management of sickle cell anaemia be established by member states with the aim of reducing morbidity and mortality associated with sickle cell anaemia. National programs need to focus on: surveillance, information dissemination, genetic counseling and testing. Other measures proposed include: offering appropriate, affordable and easily accessible emergency care to people living with sickle cell anaemia, developing capacity to evaluate impact of sickle cell anaemia programs, training of health professionals in high prevalent areas, developing holistic care within existing primary health care systems offering medical genetics services in partnership with various levels of governments and Nongovernmental organizations, promotion of relevant community education (including health counseling), promotion of effective international cooperation in combating sickle cell anaemia and support of research on sickle cell anaemia (WHO, 2006.).

Recently, in the country there has been increased public sensitization about sickle cell disease from the media and the theme for the world sickle cell day held in Kenya in 2015 was “know your sickle cell status” which is a step in controlling the perpetuation of the gene.

Health education, screening for sickle cell and diagnosing the carriers of the gene is therefore essential in control of the disease as the burden will be quantified in local populations and affected persons are likely to make informed reproductive choices resulting in prevention of birth of children with sickle cell disease.

This study was conducted in adolescents attending mixed day secondary schools in Bungoma County, found in western Kenya. The focus of this study was on primary intervention involving public health education and adolescent screening to ascertain the frequency of the sickle cell trait in Bungoma County.

Testing for sickle cell was done using a rapid immunoassay diagnostic test for sickle cell disease called the Sickle Scan™.

1.2 Research problem statement

Sickle cell disease is a significant cause of morbidity and mortality in Africa, with up to 75% of cases occurring in the continent. Three hundred thousand infants are born with haemoglobinopathies in the world, 83% of which are sickle cell disorders. Of all under five deaths, 3.4% are sickle cell deaths (Modell B, 2008). Early childhood mortality from sickle cell disease in Africa is high up to 90% (Grosse SD O. I., 2011) compared to North America where 90% survive into adulthood (Quinn CT, 2010). The need for primary intervention hence becomes important involving screening early on in life for the trait to control perpetuation of the gene.

The WHO estimates the Sickle cell carrier rate at 10% to 40% in sub-Saharan Africa; these are healthy individuals who have the potential to unknowingly perpetuate the gene.

There is generally low public knowledge on sickle cell disease and it is significantly associated with psychosocial and economic burden (Marsh VM, 2011). Sickle cell trait persons in reproductive age with lack of knowledge on their sickle cell status are likely to unknowingly give birth to a sickle cell disease offspring.

The average cost for managing a patient with the disease in an outpatient setting in rural Kenya is estimated to be KES 13,800 (USD 138) annually. In settings where majority of population live under abject poverty, the cost of managing a sickle cell disease patient becomes significant in the household budget and is likely to affect the survival of an affected individual. (Amendah DD, 2013).

There is limited epidemiological data on sickle cell disease around the globe and most nations with significant burden of sickle cell disease do not have control programs, Kenya included (WHO, 2006.).

Adolescents in secondary schools are just about to become adults and likely to plan a reproductive lifestyle. There is therefore need to educate and test this group.

1.3 Justification

Transmission of the sickle cell disease is autosomal recessive. Those with the trait are mostly healthy individuals who can unknowingly contribute to the birth of the children with homozygous state who present with complications that result in increased morbidity and mortality with high treatment costs and psychological burden. Knowledge on sickle cell status is likely to inform decision making on reproductive lifestyle with resultant control of transmission of the sickle gene.

In countries where primary intervention strategies have been adopted, for example in North America, there has been improved survival in the population with reduction of sickle cell related deaths. (Chakravorty S, 2015)

Prevalence studies for sickle cell are therefore important to quantify burden of disease so that strategies can be put in place to control the transmission of the gene and more so the SS combination (homozygous state) which is fatal.(Piel FB H. S., 2013)

Adolescents in secondary school are able to understand information, are about to become adults and are accessible. They are therefore a suitable group to screen as they approach adulthood so that they can make decisions in future regarding their

reproductive life basing on their sickle cell status. This will contribute towards primary prevention of sickle cell disease by reduction in the number of children born with the homozygous combination.

Knowledge on sickle cell disease and status among adolescents is likely to inform relationship decisions and eventual spouse choice in their future where they will be adults and are likely to reproduce. Persons with sickle cell trait and knowledge about sickle cell disease are likely to pair with persons who have normal haemoglobin and prevent the possibility of giving birth to children with sickle cell disease.

1.4 Research question

What is the prevalence of sickle cell trait among adolescents in Bungoma County?

1.5 Broad objective.

To determine the prevalence of sickle cell trait among adolescents in Bungoma County.

1.6 Specific objectives.

1. To determine the prevalence of sickle cell trait among adolescents attending mixed day secondary schools in Bungoma County.
2. To determine the haemoglobin variants among adolescents attending mixed day secondary schools in Bungoma County.
3. To test the association between the demographic characteristics and sickle cell trait among adolescents attending mixed day secondary schools in Bungoma County.

CHAPTER TWO

LITERATURE REVIEW

This chapter describes the general information on sickle cell disease, the prevalence of the trait, haemoglobin variants and how to determine them using various tests and the demographic associations of the Sickle cell trait. The WHO intervention strategies for SCD will also be highlighted.

2.1 General information on sickle cell disease

Sickle cell disease is a genetic disorder of the haemoglobin molecule whose proximate cause is a mutation of the β-globin chain of hemoglobin. (Ansong, Akoto, Ocloo, & Ohene-Frempong, 2013; Kreuels et al., 2009).

The haemoglobin molecule is constituted by four globin chains; fetal haemoglobin (HbF) which has two alpha and two gamma chain (a_2y_2) and adult haemoglobin (HbA) has two alpha and two beta chains ($a_2\beta_2$)(Domínguez et al., 2013). Genes in the α-globin and β-globin clusters on chromosome 16 and 11 respectively and control the production of globin chains(Iyer, Sakhare, Sengupta, &Velumani, 2015). Point mutation result in haemoglobin gene variants which fall into two broad groups – structured variants in which there is a change in the sequence of amino acid producing unusual haemoglobin (haemoglobinopathy) and thalassaemia in which there is a lower or abolished production of globin chains. Sickle cell anaemia is caused by a point mutation in the β-globin chain of haemoglobin causing the hydrophilic amino acid glutamate to be replaced by the hydrophobic amino acid valine at the sixth position(Malowany& Butany, 2012).

More than 300,000 children are born annually with hemoglobinopathies globally, of which 83% are sickle cell disorders (Modell & Darlison, 2008).

Also known as sickle cell anemia; it is characterized by an abnormality in oxygen carrying hemoglobin molecule in red blood cells (Bowers, Pepple, & Reid, 2008). It is one of the most common hemoglobinopathies in Africa, Middle East and India. However, it is now found globally with an increasing incidence in Europe and North America due to increasing migration rates from areas that have high prevalence (Stuart & Nagel, 2004).

Sickle cell disease is part of the broader haemoglobinopathies which are disorders of haemoglobin structure. In this disease, red blood cells assume the abnormal sickle shape and become rigid under conditions of low oxygen such as dehydration, acidosis and infection (Malowany& Butany, 2012).

There are a variety of genotypes of the sickle cell syndrome. There is homozygosity for the mutation that cause HbS – sickle cell anaemia (SCA) that is also referred to as 'HbSS' or 'SS disease'. In the heterozygous state, where there is one sickle gene and one normal haemoglobin gene, the result is a condition referred to as 'Hb AS' or sickle cell trait (SCT). Other forms of sickle cell disease are compound heterozygous states where there is a copy of the mutation that cause HbS and a copy of another abnormal haemoglobin allele. Examples include sickle-haemoglobin C (HbSC), sickle beta-zero thalasaemia (HbSβ°), sickle beta-plusthalasaemia (HbSβ^{+}), sickle-haemoglobin D-Punjab (HbSD-) among others. The different types of sickle cell syndromes exhibit varying degrees of severity of the clinical features(Malowany& Butany, 2012).

The allele responsible for sickle cell anaemia is autosomal recessive and is found on the short arm of chromosome 11.Usually, the parents of an individual with HbSS are heterozygote and therefore carry one HbS allele (HbAS) and are asymptomatic thereby being referred to as carriers or have the sickle cell trait (Malowany J.I., 2012). However,in certain populations, because of the high rate of HbS carriers(HbAS), it is possible that the parent may be homozygous (HbSS) or a compound heterozygote (HbSC). If both parents are heterozygous for the HbS mutation (HbAS), there is a 25% chance per pregnancy of having an offspring with Sickle cell disease (HbSS) and 50% chance of the offspring being a carrier (HbAS) and a 25% chance of the offspring having the normal Haemoglobin (HbAA). If one parent is homozygous (HbSS) and the other parent is heterozygous (HbAS), there is a 50% chance for every pregnancy of having an affected offspring (HbSS) and a 50 % chance of having a carrier (HbAS). If one parent is homozygous (HbSS) and the other is normal (HbAA), then every pregnancy is likely to result into carrier state (HbAS). If both parents are homozygous (HbSS) then every pregnancy is likely to result into a sickle cell disease offspring (HbSS). Persons with the trait (HbAS) or the disease (HbSS) should pair with normal persons (HbAA) in order to avoid the likelihood of getting an affected offspring (HbSS).

Various complex interactions of the sickle red cells with the endothelium and other blood cells have been described that could contribute to the pathophysiology of sickle cell disease which involve adhesion molecules and production of pro-inflammatory mediators(Manwani& Frenette, 2013).

The clinical manifestation of sickle cell disease is varied with any organ being affected. These are classified as: Vaso-occlusive and hematologic. Vaso-occlusive

manifestations include pain, acute chest syndrome, splenic infarction, stroke and avascular necrosis of joints being prominent. Hematologic manifestations include episodes of severe anemia, leg ulcers and pulmonary hypertension (Ansong et al., 2013; Kato, Gladwin, & Steinberg, 2007).

With early diagnosis and treatment, it is possible to ameliorate the course of the disease and ultimately improve patient survival. Better survival rates and improved quality of life have been attributed to prophylactic penicillin, immunizations, comprehensive care and parental education about the serious sickle cell disease complications (Ndefo, Maxwell, Nguyen, & Chiobi, 2008; Tsaras, Owusu-Ansah, Boateng, & Amoateng-Adjepong, 2009).

Although persons with sickle cell trait are usually asymptomatic, they could potentially be predisposed to complications such as: gross hematuria, increased urinary tract infection rates in women, complications of hyphema, splenic infarction with altitude hypoxia or exercise and life threatening complications of exercise (Eichner, 2007). Hematuria has been recognized as the most common manifestation of sickle cell trait (Ahmed & Ibrahim, 2006).

2.2 Prevalence of HbAS.

Globally, sickle cell trait is present among 300 million individuals with the highest prevalence of 30-40% in sub-Saharan Africa (Kato et al., 2007). According to the WHO, the sickle cell trait is widespread reaching its highest prevalence in parts of Africa as well as people with origins from equatorial Africa, the Mediterranean basin and Saudi Arabia. In Africa the prevalence of the sickle cell trait is highest between latitudes 15 degrees North and 20 degrees South, ranging between 10% and 40%of the population in some areas. The prevalence decreases to 1% and 2% in north Africa and less than 1% in southern Africa. Where the prevalence is above 20%, the disease affects up to 2% of the population.

Prevalence studies of the Sickle cell trait done in East Africa have yielded results that fall within the 10% to 40% estimated prevalence for Sub-Saharan Africa. In Uganda the prevalence of the trait is 13.3% (Ndeezi et al., 2016). In western Kenya HbAS prevalence is 17.1% (Suchdev PS, 2014). These studies were done in children less than18 and 35 months respectively. In the Democratic Republic of Congo, the prevalence of sickle cell trait in screened newborns was 23.3% (Agasa B, 2010).

In Saudi Arabia, about 4.2% of the population carries the sickle cell trait (Jastaniah, 2011).The prevalence of Sickle cell trait in India ranges from 9.4% to 22.2% (Awasthy et al., 2008).

In Lebanon the prevalence of the sickle cell trait is 0.49%. The participants had a mean age of 24.9 (± 15.6) years. This overall prevalence was higher than a previous Lebanese study in Beirut (Khoriaty et al., 2014) found a prevalence of 0.27% for all hemoglobin variant

The prevalence of Sickle cell trait was 1.5% among infants screened in 44 states of the United States of America (Ojodu, Hulihan, Pope, Grant, & Centers for Disease Control and Prevention (CDC), 2014). In a Brazilian national neonatal screening program study established a prevalence of 1.1-9.8% (0.8-60 per 100, 000 live births) for sickle cell trait in different regions of Brazil (Lervolino et al., 2011). In Italy, sickle cell trait prevalence was 0.7% between 2007and 2009 (Ballardini et al., 2013).

Most of sickle cell trait prevalence studies have been conducted among children below five years of age. However, there are few studies that have been done among older populations. In an 8-year cohort study in Kilifi-Kenya, the prevalence of sickle cell trait was found to be 14.6% (313/2141) (Wambua, Mwacharo, Uyoga, Macharia, & Williams, 2006). Studies in Nigeria conducted among university students have

reported that the prevalence of sickle cell trait ranges between 19-24% (Adeyemo & Soboyejo, 2006; Jeremiah, 2006).

2.3 Prevalence of HbSS.

From previous studies, majority of HbSS are witnessed among children below five years (Suchdev, Ruth, Earley, Macharia, & Williams, 2014). However, studies among older age groups show little or no HbSS prevalence. This is evidenced by both Kenyan (Wambua, Mwangi, et al., 2006) and Nigerian studies (Adeyemo & Soboyejo, 2006; Jeremiah, 2006). In Western Kenya, the prevalence of HbSS among children between 6-35 months was 1.6% (Suchdev et al., 2014); however, in Kilifi–Kenya, the prevalence was 0.001% among older children(Wambua, Mwacharo, et al., 2006). In Nigeria, the prevalence of HbSS among older participants ranged between 0-1.3% (Adeyemo & Soboyejo, 2006; Jeremiah, 2006).

A study in Uganda reported the prevalence of HbSS as 0.7%(Ndeezi et al., 2016).This was done in children less than18 months.

In a study done in the Democratic Republic of Congo, the prevalence of HbSS in screened newborns was reported as 0.96% (Agasa B, 2010).

In the United Kingdom, one baby in every 2000 is born with Sickle cell disease according to the National Health Service (NHS). In France, the overall birth prevalence was found to be 1 in 2415 (Bardakjian et al., 2000).In Saudi Arabia, about 0.26% of the population has sickle cell disease(Jastaniah, 2011).

From these studies it is clear the sickle cell disease burden is heaviest in Africa. Reducing prevalence with increasing age is an indication of mortality of those with the SCD.

2.4 Prevalence of other haemoglobin variants

Whereas the sickle cell gene is the most common abnormal haemoglobin variant, there exists other abnormal haemoglobin variants. The commonest abnormal haemoglogin variant other than the HbS is the HbC. HbC is more limited than HbS to geographical areas in the West and North West Africa with a prevalence of up to 15% (Piel et al., 2013). However, there has been a low prevalence along the corridor between West Africa and Egypt due to human migration(Williams & Weatherall, 2012). In a West African study; HbC is thought to have originated along Niger river as a result of the founders' effect (Cataldo, 2012; Williams & Weatherall, 2012). The prevalence was as high as 40% in Northern Ghana, up to 50% in Northern Ivory Coast, 20% in Togo and Benin, less than 1% in Central Africa and nonexistent in East Africa (Bachir & Galacteros, 2004)

From studies described above, all forms of HbC gene combinations namely HbAC, HbCC, HbSC were grouped together to give the HbC variants prevalence

There is paucity of data on prevalence of other described haemoglobin variants like the HbD- Punjab variant.

2.5Association between demographic characteristics and sickle cell trait

The prevalence of the sickle cell trait is highest between latitudes 15 degrees North and 20 degrees South. The explanation as to why the sickle cell trait is highly prevalent in the tropics has been linked to its association with protection against malaria(Piel et al., 2013). In Western Uganda, it was observed that the prevalence of sickle cell trait was significantly higher in Bundibugyo (13.4%) compared to Mbarara/Ntugumo (3%) regions (Okwi et al., 2010). This was attributed to a high prevalence of malaria in Bundibugyo and less intermarriage (consangunity).

Being an inherited disease, the likelihood of being a carrier, or having the sickle cell disease increases with known family history of sickle cell disease (Malowany& Butany, 2012). The sickle cell gene frequency is beginning to affect other parts of the world previously not known to have sickle cell disease as a result of migration and intermarriage. Previous studies have shown that there is no significant association between sickle cell trait and gender (Adeyemo & Soboyejo, 2006; El Ariss, Younes, Matar, & Berjaoui, 2016; Jeremiah, 2006; Piel et al., 2013; Wambua, Mwacharo, et al., 2006).

2.6 Laboratory diagnosis of sickle cell gene status.

Laboratory diagnosis is based on identification of HbS and the absence or reduction in the quantity of HbA in red blood cells. Screening tests available commonly in laboratories in Africa include the Sodium metabisulphite sickling tests and sickle solubility tests. Confirmatory tests confirm the phenotype. Possible phenotypes in sickle cell disease include SS,AS,SC,S$\beta-$ thalassemia. Tests commonly used in describing the phenotypes include Hb electrophoresis (HBE), Isoelectric focusing(IEF) and High performance liquid chromatography (HPLC)(J. Makani, 2013).A newer point of care rapid diagnostic immunoassay test has been developed which has comparable sensitivity to HBE,IEF and HPLC(Julie Kanter, 2015).

The sickling test for screening is based on the principle that sodium metabisulphite creates hypoxic states for red blood cells and causes them to sickle and this can then be visualized under a microscope. The sickling solubility test is based on the relative insoluble state of HbS when combined with a reducing agent such as sodium dithionite.The sensitivity of the sickling test is 65% and that of solubility test is 45%.(Okwi A.L, 2010). The sickling test will be positive in persons with both HbAS and HbSS. However, it cannot differentiate HbAS from HbSS.

Confirmatory tests are based on the principle that different Hb isoforms have different ionic charge which makes them migrate with different velocities in an electric field. High performance liquid chromatography uses cation exchange chromatography to identify haemoglobin variants and has the advantage of the ability to quantify Hb variants. Isoelectric focusing is a qualitative test used to separate proteins based on their isoelectric points which refers to where the net charge of the protein is zero, the method is similar to Hb cellulose electrophoresis in principle only that it forms sharper bands with better resolution. Isoelectric focusing has advantage over Hb electrophoresis in that it can detect more Hb variants and requires no chemical reagents. However. Isoelectric focusing cannot quantify Hb variants whereas Hb electrophoresis can. In terms of sensitivity; High performance liquid chromatography and Isoelectric focusing are comparable to Hb Electrophoresis with sensitivities more than 99%. Important to note is that High performance liquid chromatography, Isoelectric focusing and Hb Electrophoresis have a long turn around time, require machines for analysis and highly skilled trained personnel to operate them(J. Makani, 2013).

An emerging diagnostic tool is the rapid immunoassay testing that is based on antigen antibody interaction, the currently available kit based on this principle is the Sickle Scan™ which only requires about five microlitres of blood put in a buffer pretreatment to lyse red blood cells and release haemoglobin. The released haemoglobin is then allowed to travel on a special cartridge and react with specific antibodies producing blue lines for specific Hb variants A, S and C. A control line is also visualized. This test has comparable sensitivity to HBE, IEF and HPLC at more than 99%. This test kit was designed for low income settings, requires minimal training, does not require specialized machines, is not labour intensive and results are

available within five minutes at the point of care. Its pitfall is that it is limited to diagnosing HbA, HbS and HbC variants only and is not recommended in infancy because of the possibility of interference of results by fetal haemoglobin.(Julie Kanter, 2015).

Prevalence studies for the trait can therefore be conducted using any of the mentioned tests with sensitivity above 99%. This study used the rapid immunoassay method for testing participants.

2.7 Control of sickle cell disease.

The WHO intervention strategy for sickle cell disease lists primary intervention strategies as public health education and genetic counseling together with making appropriate marriage choices to avoid giving birth to children with sickle cell disease. Secondary intervention involves early diagnosis through newborn screening and offering immunizations, good nutrition, folate administration and prophylaxis against infections to prevent complications. Tertiary intervention involves management of complications of sickle cell disease and rehabilitation including analgesia, hydration and blood transfusion.

Globally, most intervention strategies are focused on newborn screening. In Africa the program is being established in Ghana, Liberia, Nigeria and Tanzania in collaboration with the Association of public health laboratories (APHL) and Centers for Disease Control(CDC). Majority of other countries in sub-saharan Africa with high sickle cell burden have not taken this important secondary intervention strategy. In the United States, the National sickle cell control act of 1972 provided a platform for setting up sickle cell disease clinics that conduct testing, genetic counseling and education on sickle cell. This strategy has improved survival in the United States (Chakravorty S, 2015). Other secondary interventions include immunizations against encapsulated organisms, prophylaxis against malaria and other infections by penicillins, folate is

also recommended because of the high turnover of red blood cells and hydroxyurea administration to increase levels of fetal haemoglobin which leads to prevention of crises and improves survival. Continuous health education regarding sickle cell disease and triggers of crisis is also important. These strategies are essential in improving survival of an affected individual. Tertiary control involves treatment of crises and sickle cell disease complications when they arise.

Primary intervention is usually accompanied by ethical concerns especially in married couples and pregnant mothers; involving whether to annul a marriage or opt for other options like birth control and adoption. In pregnancies with the fetus affected by sickle cell disease, termination of pregnancy raises lots of ethical questions. A study in Cameroon found the acceptability of prenatal diagnosis in doctors to be 78.7% and in parents who previously had an affected child to be 89.8%. Despite termination of pregnancy option not being readily available, up to 62.5% would terminate the affected pregnancy if given the option (Ambroise Wonkam, 2015.). In Nigeria, a study on premarital couples found that among couples, up to two thirds would call off the marriage if there was risk of their offspring inheriting sickle cell anaemia (Nnaji G.A, 2013).

The best timing for primary intervention seems to be at premarital level involving counseling couples before marriage and at secondary school level to help affected individuals choose appropriate partners in their reproductive life. A good example of screening is in Jamaica where secondary school students were tested to know their sickle cell status. The screening program targeted 15 to 19 year olds in the fifth and sixth forms. Education on sickle cell in this group increased demand for testing over a six year academic period beginning 2007/8 from 56% to 92% (Karlene Mason, 2015).

Overall, newborn screening may contribute to prevention of birth of children with HbSS; there is a probability of the affected children making informed spouse choices when they grow up into adults with knowledge of their sickle cell gene status. It is therefore important to have genetic counseling and screening programs accompanied with public education on sickle cell disease so that control of the disease can be successful through a community strategy.

CHAPTER THREE

METHODOLOGY

3.1 Study site

This study was conducted in Bungoma County, which is county number 39 in the Republic of Kenya. It covers an area of 3,032 Km^2. Bungoma county borders Trans-Nzoia County to the Northeast, Kakamega County to the East and Southeast and Busia County to the West and Southwest. It also borders the republic of Uganda to the Northwest.

Bungoma county is made up of nine constituencies (geopolitical administrative regions)namely:Bumula, Kanduyi, Kabuchai, Sirisia, Kimilili, Tongaren, Mt. Elgon, Webuye East and Webuye West.The population as at 2009 census was 1,374,627, of these Males were 671,222(48.8%) and Females were 703,405 (51.2%). Those in the 10 to 20-year age bracket were 347,875, of these, 173,570 were Females(49.9%) and174,305 were Male(50.1%).

The County has 275 public and 12 private secondary schools, with a secondary eligible age population of 150,738, of which 75,597(50.2%) are boys and 75,141(49.8%)are girls. There are 189 mixed day secondary schools in Bungoma County. The county has 136 health facilities of which 11 are hospitals, 4 nursing homes, 16 health centers, 78 dispensaries, 27 clinics and 134 community units. (County, 2015)

3.2 Study Population

The study populations were adolescents attending mixed day secondary schools in Bungoma County.

3.3 Study design

This was a descriptive cross-sectional study.

3.4 Sample size

The Sample size were calculated using the Fischer's formula. Prevalence of sickle cell trait used in this study was 17.1% (Suchdev PS, 2014) among a paediatric population aged less than 35 months. This fell within the World Health Organization of 10 to 40% range, and it was done in Western Kenya of which Bungoma is part of. Since sickle cell trait children are likely to have a normal healthy life this could still be representative of older age groups in particular the adolescents who were the target of this study. The prevalence of the trait in this study was similar to the prevalence in Eastern Uganda which borders western Kenya (Andrew L. Okwi, 2010).

Confidence interval at 95%,

Z of 1.96

Margin of error (e) at 5 %(0.05) .

Application of formulae: $N = \frac{Z^2P(1-P)}{e^2} = \frac{1.96^2 \text{ X } 0.171 \text{ X}(1- 0.171)}{(0.05)^2}$

Sample size from formulae application yields 218 participants.

3.5 Eligibility

3.5.1 Inclusion criteria

i. Students attending mixed day secondary schools in Bungoma County.

ii. Aged between 10 and 19 years.

3.6 Sampling procedure

Stratified simple random sampling was adopted. Bungoma County was stratified into the nine constituencies. A mixed day secondary school was then picked by simple random sampling (by a neutral party) from each constituency, giving a total of nine secondary schools. Twenty five students per school who met the eligibility criteria were then randomly selected from the school registers of the randomly selected schools representing the nine constituencies. Adolescents were sourced from mixed

day secondary schools to ensure that both male and female gender was equitably represented.

3.7 Execution of the study

The study was conducted over a six month (January to March 2017, May to July 2017) period that coincided with the school calendar. The research assistants included a nurse and a laboratory technician who were trained on counseling and testing for sickle cell disease using the sickle scan™ rapid test. The Research assistants were under the supervision of the Principal investigator. The 9 randomly selected schools were Bungoma Muslim Secondary School(Kanduyi constituency), St. Mark's Kipchiria Secondary School(Mt.Elgon constituency), D.E.B Lunyu Secondary School(Tongaren costituency), Hon. Moses Wetangula Mikayu Friends Secondary School(Kabuchai constituency), Sitikho Friends Secondary School(Webuye West constituency), Lugusi Secondary School (Webuye East constituency), Sikhendu Mixed Secondary School (Kimilili constituency), Chebukuyi ACK Secondary School (Sirisia constituency) and Chiliba Secondary School (Bumula constituency). Public health education on SCD control was offered to 2389 students in the 9 schools by the Principal Investigator assisted by the Research Assistants, 2248 met eligibility criteria. A total of 225 eligible participants were selected randomly from the school registers and consented. Pretest counseling was done followed by testing for sickle cell gene status using a point of care(POC) device (The Sickle scan™) where capillary blood was obtained from a finger prick under sterile conditions, mixed with buffer and introduced to a cartridge, results were ready within five minutes. Post test counseling was then offered. Counselling was offered in make shift screened rooms. Demographics and test results were documented.

3.8 Data collection instruments

A data collection tool (Appendix II) collecting demographic characteristics and screening test results were used. The demographic data of interest were age, gender, ethnicity/sub-ethnicity, constituency, sub-county, location, village and family history of sickle cell disease. The screening test results was documented as the various haemoglobin variants.

3.9 Data management.

Data collected was cleaned, entered into Microsoft Excel and exported to SPSS version 23 and presented in cross tabulation and histograms.

The data was backed up to prevent data loss.

3.10 Data analysis

Mean and Median was used to describe continuous data and proportions/percentages for categorical data.

Logistic regression was applied to generate risk ratios and associations were tested using the pearson chi square test.

Confidence interval set at 95% and level of significance p-value <0.05.

3.11 Ethical considerations.

Approval for the study was obtained from the Moi University School of Medicine and Moi Teaching and Referral Hospital Institutional Research and Ethics Committee.

Permission for community entry was sought from the Bungoma County Director of medical services and the Bungoma County Director of Education. Permission from the participating schools was sought from the Parents Teachers Association and Board of Governors through the school principals.

Health talks were given to the participating schools to inform them about sickle cell disease, its transmission and importance of getting tested and knowing their sickle cell status.

Informed written consent and assent (Appendix III, IV, V and VI) from parents of adolescents less than eighteen years old was obtained. This was facilitated on a convenient day organized by the participating schools through the school principals. For parents unable to be met at school but having access to a mobile phone, consent forms were delivered to them by their children and a follow up explanation through mobile phone communication was done by the research team on what the study was all about. Those above eighteen years of age gave informed written consent by themselves. Those under eighteen years required parental consent and they gave assent before participating in the study.

Adequate pre and post test counseling to offer reassurance and allay any anxieties, psychological support and medical advice was offered to participants. This counseling was offered by trained research assistants. (Appendix VII)

There was strict adherence to laboratory standard operating protocols for carrying out the screening test (Appendix VIII). Biohazard waste was safely disposed. This was achieved through training of the research assistants who were recruited in this study.

Those who were not sampled but wished to be tested were directed on how to get access to counseling and testing.

Confidentiality was maintained when handling participants, their results and any other data collected. This was achieved through data coding, encryption and saving them in password protected computers.

CHAPTER FOUR

RESULTS

4.1 Demographic characteristics.

A total of 225 adolescents from mixed-day secondary schools in 9 constituencies from Bungoma County, Kenya were enrolled into this study. Each constituency contributed 25 participants. All of the sampled participants agreed to participate. Of these, 116 (51.6%) were male while 109 (48.4%) were female. The participants' age ranged between 13 to 19 years with a median age of 17 years and a mean age of 16.75 years (SD±1.485) as shown in Table 1. Of the 225 participants 12(5.3%) had a known family history of sickle cell disease; one had a sibling affected, nine had an affected cousin and two had non-specified relatives with sickle cell disease as shown in Table 1.

Table 1: Demographic characteristics.

Variable	Values
Age (years)	
Minimum	13
Maximum	19
Median	17
Mean	16.75 (SD 1.485)
Gender	
Male	116 (51.6%)
Female	109 (48.4%)
Family History of SCD	
Yes	12 (5.3%)
No	213 (94.7%)
If yes to Family History of SCD	
Sibling	1 (8.3%)
Cousin	9 (75%)
Others	2 (16.6%)

4.2 Prevalence of sickle cell trait among adolescents in Bungoma County.

The study found that the prevalence of sickle cell trait (HbAS) in Bungoma county between January and July, 2017 was 18.7% (95% CI: 0.14-0.24); whereby 42 of the 225 adolescents sampled had a sickle cell trait.

The study further found that Bumula constituency had the highest (36%; 9/25) sickle cell trait burden, almost double the average prevalence. Other constituencies that had higher than overall prevalence were Webuye West (28%; 7/25), Mt. Elgon (20%; 5/25) and Kabuchai (20%; 5/25). The least burden was observed in Tongaren constituency (4%; 1/25) as shown in Figure 1.

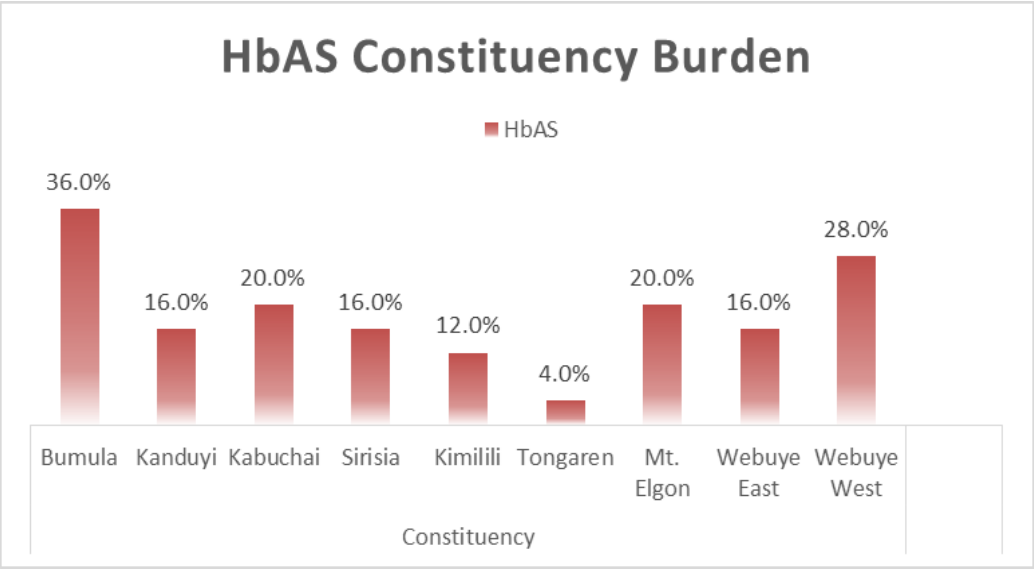

Figure 1: HbAS Constituency Prevalence

4.3 Haemoglobin variants among adolescents in Bungoma County.

The haemoglobin variants determined among adolescents attending mixed day secondary schools in Bungoma County were: HbAA (81.3%; 183/225),HbAS (18.7%; 42/225). The other variants HbSS, HbAC, HbCC and HbSC were 0% (0/225) with no invalid results as shown in table 2.

Table 2: Hemoglobin Variants among adolescents in Bungoma County

Hb Variant	Number/225	Percentage (%)
HbAA	183	81.3
HbAS	42	18.7
HbSS	0	0
HbAC	0	0
HbCC	0	0
HbSC	0	0
Invalid	0	0
Total	**225**	**100%**

4.4. Association between demographic characteristics and sickle cell trait

The study tested the association of the following demographic characteristics: gender, geographical area (constituency) and family history of sickle cell disease with sickle cell trait.

4.4.1 Gender

Of the male participants (n= 116) enrolled into the study; 20.7% (n=24) had a sickle cell trait while among the female participants (n=109), 16.5% (n=18) had a sickle cell trait as shown in table 3.

Table 3: Gender versus Sickle Cell Test Results

		Sickle Cell Test Results		Total
		HbAA	**HbAS**	
Gender	**Male**	92(79.3%)	24(20.7%)	116(100%)
	Female	91(83.5%)	18(16.5%)	109(100%)
Total		183(81.3%)	42(18.7%)	225(100%)

Being of the male gender conferred a 1.253 (95% CI: 0.721-2.177) relative risk of sickle cell trait, compared to being female. However, this was not statistically significant (p-value = 0.422) as shown in table 4.

Table 4: Test of Association of Gender and Sickle Cell Trait

Gender (Male / Female)	RR (Relative Risk)	95% Confidence Interval		Pearson Chi-square Test of association
		Lower	Upper	p-value
For cohort Sickle Cell Test Results = HbAS	1.253	.721	2.177	0.422
N of Valid Cases	225			

4.4.2Geographical Area (Constituency)

Residents of Bumula constituency had a two-times likelihood of having sickle cell trait compared to non-Bumula constituency residents, with a risk ratio of 2.182 (95% CI: 1.187-4.010). This was statistically significant (p-value=0.018) as shown in table 5.

Table 5: Association between Constituency and Sickle Cell trait

		Sickle Cell Test Results			
		HbAA	**HbAS**	**RR (95% CI)**	**P-value**
Constituency	**Bumula**	16	9	**2.182 (1.187-4.010)**	**0.018**
	Kanduyi	21	4	0.842 (0.382-2.161)	0.717
	Kabuchai	20	5	1.081 (0.468-2.495)	0.856
	Sirisia	21	4	0.842 (0.382-2.161)	0.717
	Kimilili	22	3	0.615 (0.205-1.845)	0.364
	Tongaren	24	1	0.195 (0.028-1.356)	0.046
	Mt. Elgon	20	5	1.081 (0.468-2.495)	0.856
	Webuye East	21	4	0.842 (0.382-2.161)	0.717
	Webuye West	18	7	1.600 (0.797-3.212)	0.204
Total		**183**	**42**		

4.4.3 Family history and Sickle Cell trait

Among all the study participants, 5.3% (n=12) had a family history of sickle cell disease. Of those with family history of sickle cell disease, 50% (n=6) had sickle cell trait. Of those without a family history of sickle cell disease, 16.9% (n=36) had sickle cell trait, as shown in table 6.

Table 6: Family History of Sickle Cell Disease versus Sickle Cell Test Results

		Sickle Cell Test Results		Total
		HbAS	HbAA	
Family History of Sickle Cell Disease	**Yes**	6 (50%)	6 (50%)	**12 (100%)**
	No	36 (16.9%)	177 (83.1%)	**213 (100%)**
Total		**42 (18.7%)**	**183 (81.3%)**	**225 (100%)**

Adolescents with a family history of sickle cell disease were three times more likely to have the sickle cell trait with a relative risk (RR) of 2.958 (95% CI: 1.561-5.607) than those without a family history, this was statistically significant (p-value = 0.004) as shown in table 7.

Table 7: Test of association for Family History of Sickle Cell Disease and Sickle Cell Trait

History of Sickle Cell Disease (Yes / No)	**RR (Relative Risk)**	**95% Confidence Interval**		**Pearson Chi-square Test of association**
		Lower	Upper	p-value
For cohort Sickle Cell Test Results = HbAS	**2.958**	1.561	5.607	**0.004**
N of Valid Cases	225			

CHAPTER FIVE

DISCUSSION

Inherited haemoglobin disorders (sickle cell disorders and thalassaemia) were originally characteristic of the tropics and subtropics but are now common worldwide due to migration. However, globally they are still common among people whose ancestors lived in tropical and sub-tropical sub-Saharan regions.

The world health organization (WHO) recognized sickle cell disease as global public health problem and urged member states to come up with national control programs for sickle cell disease among them supporting community awareness programs and research on sickle cell disease; this study is a step towards the implementation of WHO primary intervention strategies which include public health education on sickle cell disease, genetic counseling and testing and advice on reproductive choices (World Health Organization, 2005). Where Programmes for Sickle cell disease control exist, there has been notable success with increased survival of persons with Sickle cell disease and reduced birth of children with Sickle cell disease (Quinn CT, 2010). Primary interventions are aimed at preventing birth of children with sickle cell disease while secondary and tertiary interventions are designed to improve the quality of care and life of those born with sickle cell disease.

It is high time this region of Africa embraces Sickle cell control interventions and this study is a step in the right direction since it quantifies the burden in this region.

This chapter will discuss the findings from this study which can then be used to direct resources for necessary interventions.

5.1 Prevalence of sickle cell trait among adolescents in Bungoma County.

In this study, the prevalence of sickle cell trait (HbAS) among mixed-day secondary school students from Bungoma County between January and July, 2017 was 18.7%. This is almost similar to the findings of 17.1%; found in Western Kenya villages of Nyando, Kisumu County among 858 children aged 6-35 months (Suchdev et al., 2014). This could be attributed to geographical similarities of the study settings (as both were conducted in Western Kenya's - malaria holo-endemic region) with a similar study design (cross-sectional study design). The slight difference in the prevalence values could be attributed to the target population (children below 3 years) and a larger study population (n=858). However, findings were higher than a Kenyan 8-year cohort study in Kilifi which found a HbAS frequency of 14.6% (313/2141) (Wambua, Mwacharo, et al., 2006). This could be attributed to the differences in study objectives, sample size and study methodology. In the Kilifi study, the main objective was to determine the proportion of co-inheritance of αthalassaemia and sickle cell trait among children who survived after being monitored for hospital admission from malaria and other diseases (Wambua, Mwacharo, et al., 2006).

The sickle cell trait prevalence in this study is higher than in Eastern Uganda (Okwi et al., 2010). In Eastern Uganda, which borders Bungoma County in Western Kenya; the prevalence of sickle cell trait was found to be 17.5%. This difference could be due to study methodological differences such as sample size (n=286), study population age differences (6-60 months) (Okwi et al., 2010).

The findings of this study are different from a retrospective study conducted in Beirut-Lebanon between 2002 and 2014 from laboratory records of 184,105 study subjects, 899 of them were found to have sickle cell trait with a prevalence of 0.49% (95% CI, 0.46-0.52%). This could be attributed to differences in study settings and

geographical locations. Sickle cell trait has been associated with protective benefits against falciparum malaria (Williams et al., 2005) Many malaria endemic regions have equally high sickle cell prevalence; however, Lebanon eradicated malaria disease in the 1950s, hence the possible explanation to the very low prevalence of HbAS(Khoury, Asmar, Yeretzian, & Nassif, 2012).

It is important to note that despite the methodological differences, all the East African studies cited above reported prevalence values falling within the WHO estimates of 10% to 40% for Equatorial Africa. This region of Africa also has a high burden of the homozygous state (HbSS) which usually presents with lifelong symptoms (Grosse et al., 2011). In this region the WHO recommends heightened interventions for Sickle cell disease control. The absence of primary prevention strategies in this group will lead to continued unknowing perpetuation of the sickle cell gene; with chances of unknowingly getting offsprings with sickle cell disease if persons with the sickle cell trait pair up unknowingly and have biological children (Modell B, 2008).

5.2 Haemoglobin variants among adolescents in Bungoma County.

The study determined a prevalence of 81.3% (183/225) for HbAA(normal haemoglobin). This is consistent with a Nigerian study among students of African ancestry which found a HbAA prevalence of 80.32% (Jeremiah, 2006). However, our study findings were higher than another Nigerian study conducted among students in a single university of Lagos department; which found a HbAA prevalence of 70% (Adeyemo & Soboyejo, 2006). This difference could be attributed to localization of sampling to a single university department as opposed to representative sampling of the entire university students.

The knowledge that there is naturally a high proportion of persons with normal haemoglobin (HbAA) should drive public health education and control programmes in encouraging pairing up between persons with normal haemoglobin and those with the sickle cell gene (HbAS and HbSS) so as to prevent birth of offsprings with the homozygous state (HbSS).

This study found no participant with HbSS (Sickle Cell Disease -SCD). This is consistent with a Kenyan study which found 0.001% (2/2143) (Wambua, Mwacharo, et al., 2006). The study is also consistent with a Nigerian study conducted among students that found no study participant with a sickle cell gene in homozygous state (HbSS) (Jeremiah, 2006). A comparison of these findings to early child studies like the one done in Nyando, western Kenya, which reported HbSS prevalence at 1.6%(Suchdev et al., 2014) demonstrates that as children grow up the prevalence of HbSS declines. This drop could be because of deaths from complications of Sickle cell disease. Thus the lack of adolescents with HbSS could possibly be attributed to the high mortality rate of over 90% among children under 5 years of age with a diagnosis of sickle cell disease (Grosse et al., 2011). This could point towards a poor quality of care for persons with sickle cell disease. Therefore, there is need to have primary intervention strategies for sickle cell disease put in place even as we continue to improve secondary and tertiary interventions. Secondary and tertiary interventions will improve quality of care of those affected while primary interventions will prevent occurrence of new cases of the symptomatic homozygous state.

In this study, HbC variants were non-existent. This is consistent with previous studies which found that; the geographic range of HbC is more limited than HbS as it is more centered on the West and North West Africa. However, there has been a low-prevalence along the corridor between West Africa and Egypt due to human

migration. In a West African study; HbC is thought to have originated along Niger river as a result of the founders' effect. The prevalence was as high as 40% in Northern Ghana and Burkina Faso, up to 50% in Northern Ivory Coast, 20% in Togo and Benin, less than 1% in Central Africa and nonexistent in East Africa (Bachir & Galacteros, 2004).

5.3. Association of Demographic characteristicswith sickle cell trait among adolescents in Bungoma County.

5.3.1 Gender

This study determined that the male participants had a higher proportion of sickle cell trait at 20.7% (n=24) than the female participants at 16.5% (n=18). This is different from a Lebanese study (El Ariss et al., 2016) conducted in Beirut, where the proportion of sickle cell trait was lower among males (0.35%) compared to females (0.62%) despite equal number of male and female study participants. This difference in proportions could be first attributed to the very low prevalence of sickle cell trait in Lebanon(Khoriaty et al., 2014). Both studies did not find gender as a significant association with the sickle cell trait.

This study's findings also differed from a Nigerian study. In the Nigerian study (Adeyemo & Soboyejo, 2006), the proportion of males with sickle cell trait (20.8%;n=11) was lower compared to females (28.9%; n=28). This difference could be attributed to gender variations in study participants sampling proportions. In the Nigerian study, the female participants accounted for 64.7% (n=97) of the entire study population while in this study females accounted for 48.4% (n=109) of all study participants. Among the male participants, the Nigerian study had a lower proportion of male participants (25.3%; n=53) while in this study the males accounted for 51.6% (n=116).

From this study and all the studies cited in the preceding two paragraphs, gender has no association with the Sickle cell trait.

5.3.2 Geographical area (constituency):

In this study, the prevalence of sickle cell trait ranged between 4% (Tongaren Constituency) and 36% (Bumula Constituency) with an overall prevalence of 18.7% in Bungoma County. Previous studies have observed similar variations of sickle cell trait within the same geographical area. In a Ugandan study, the prevalence of sickle cell trait varied in both Western and Eastern Uganda (Okwi et al., 2010). In Eastern Uganda, sickle cell trait was highest at 17.5% (N=286) in Mbale/Sironko area. However in Western Uganda, the prevalence ranged between 3% (N=370) in Mbarara/Ntungamo and 13.4% (N=201) in Bundibugyo(Okwi et al., 2010). The authors argued that the prevalence of Bundigubyo reduced from 45% in 1949 to 13.4% in 2010 possibly due to biological (malaria control) and socio-cultural (intermarriage) factors. The stratified differences of sickle cell trait prevalence in the current study could be possibly explained by both biological and sociocultural factors.

This study further found that coming from Bumula was significantly associated (p-value= 0.018) with having the sickle cell trait compared to coming from other constituencies in Bungoma county. This was similar to the Ugandan study where in Western Uganda. Coming from Bundigubyo was significantly associated (p-value=0.001) with having the sickle cell trait compared to coming from Mbarara/Ntungamo area (Okwi et al., 2010). This could be possibly attributed to high malaria transmission in Bundigubyo and Bumula constituency; or the high levels of consanguinity and the founders' effect.

An understanding of the uniqueness in variation of local burdens of the sickle cell gene can direct resource allocation for intervention strategies.

5.3.3 Family history:

The study observed that having a family history of sickle cell disease was significantly associated (p-value = 0.004) with having a sickle cell trait. This is in line with genetic studies of the hereditary nature of sickle cell trait (Ndila et al., 2018; Tsaras et al., 2009).

This study also noted that despite having no family history of sickle cell disease, 16.9% of this group still had HbAS. This is a huge percentage. It therefore follows that interventions for sickle cell disease control should not only be restricted to those with a family history but also cut across all other populations of western Kenya.

5.4 Study Limitations

1. The point of care test kit was limited to diagnosing hemoglobin variants HbA, HbS and HbC.
2. The study missed out on younger adolescents (10 to 12years old) by virtue of it being done in secondary schools where most adolescents are above 12 years.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The study concludes that adolescent sickle cell trait prevalence in Bungoma county is high (18.7%) with the most common hemoglobin variant being HbAA. There was no HbSS. There was no HbC variant. Residing in Bumula Constituency and having a family history of sickle cell disease were significantly associated with the sickle cell trait.

6.2 Recommendations

1. Sensitization on sickle cell disease and Sickle cell trait should be done in Bungoma county to encourage residents to get tested and know their sickle cell gene status.
2. There is need for Bungoma County through the Ministry of Health to set up genetic counseling and testing centers for sickle cell disease and sickle cell trait where residents can get tested and be advised on how to prevent birth of children with sickle cell disease through informed reproductive choices.

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APPENDICES

Appendix I: Bungoma County.

Administrative and political units, Bungoma County

Constituencies	Sub-counties	Area (km^2)	No. of wards	No. of divisions locations		No. of sub locations
Bumula	Bumula	347.8	7	1	10	24
Kanduyi	Bungoma South	318.5	9	1	5	14
Kabuchai	Bungoma Central	232.3	4	4	12	18
Sirisia	Bungoma West	213.2	3	4	7	15
Kimilili	Kimilili	181.2	4	2	8	18
Tongaren	Bungoma North	378.4	6	2	12	23
Mt. Elgon	Mt. Elgon	956.6	6	4	16	40
Webuye East	Webuye East	161.8	3	2	6	13
Webuye West	Webuye West	242.6	3	1	5	14
Total		3,032.4	45	21	81	176

Appendix II: Data Collection Form

1. Participant No. ☐ ☐ ☐ ☐ ☐
2. Age in years. ☐ ☐ ☐ ☐
3. Gender. Male ☐ Female ☐
4. Constituency

Bumula ☐ Kanduyi ☐ Kabuchai ☐ Sirisia ☐ Kimilili Tongaren ☐ Mt.Elgon ☐

Webuye East ☐ Webuye west ☐

5. Sub County

Bumula ☐ Bungoma south ☐ Bungoma central ☐ Bungoma west ☐ Kimilili ☐

Bungoma North ☐ Mt,Elgon ☐ Webuye east ☐ Webuye west ☐

6. Location……
7. Village……..
8. Ethnicity……
9. Sub-Ethnicity……
10. Family History of Sickle Cell disease:

Yes ☐ No. ☐

11. If Yes to No. 9,Specify

Sibling ☐

Cousin ☐

Others ☐

12. Test results.

HbAA ☐

HbAS ☐

HbAC ☐

HbSS ☐

HbSC ☐

HbCC ☐

Invalid ☐

Not clear ☐

Appendix III: Swahili Consent Form.

IDHINI YA KUHUSIKA KWENYE UTAFITI WA KISAYANSI UNAOKUSUDIA KUJUA KIWANGO CHA "SICKLE CELL TRAIT" KWA VIJANA WALIOBALEGHE KAUNTI YA BUNGOMA.

Fomu hii imegawanywa katika sehemu mbili:

- Ujumbe kuhusu madhumuni ya utafitihuu.
- Cheti cha kukubali kuhusika kwenye utafitihuu.

Utapewa nakala ya fomuhii.

SEMEMU YA 1: UJUMBE KUHUSU UTAFITI.

Utangulizi.

Unaulizwa kuhusika kwa mwanao kwenye utafiti unaokusudiwa kubain ikiwango cha Sickle cell trait kwa vijana waliobaleghe kaunti ya Bungoma.Kuchagua kuhusika ni kwa hiari yako napia uko nauhuru wakujiondoa kwenye utafiti wakati wowote.

Pia unahaki wakuuliza ujumbe Zaidi kuhusu utafiti huu.

Ujumbe kuhusu ugonjwa wa sickle cell.

Ugonjwa wa sickle cell ni ugonjwa wa kuridhiwa ambayo inasababisha chembe chembe nyekundu ya damu kupata umbo ambayo inasababisha kuharibika haraka kwa chembe chembe nyekundu hatimaye mtu hupungukiwa na damu.Mtu huridhijeni.Mtu akiwa na jeni mbili huwa anakuwa na ugonjwa huu hatari ambayo inaweza hatarisha maisha, baadhi ya dalili ya ugonjwani macho ya manjano, uchungu mwilini na upungufu wa damu mwilini.Wale ambao wana jeni moja wako buheri wa afya lakini wanaweza zaa mtoto aliye na sickle cell iwapo mzazi mwengine pia akonajeni moja au mbili.

Madhumuni ya utafiti huu.

Utafiti huu unakusudia kujua kiwango cha watu katika jamii ambao wanabeba jeniya sickle cell.Huuutafitiutasaidiaserikalikutengenezamipangoyakudhibitiugonjwawa sickle cell.Ni vizuri pia wananchi kujua hali yao ya sickle cell kwani hii itasaidia katika kuzuia kueneakwa sickle cell.

Ujumbe kuhusu utafiti utakavyofanyika.

Vijana walio baleghe akiwemo mwanao wamechaguliwa na njia ya kisayansi, kitaka chofua tani kuwaeleza ujumbe kuhusu sickle cell, baadaya hapo watafiti watafanya kipimo cha damu kujua haliya sickle cell kwa mwanao. Kipimo chenyewa kina uchungu kiasi tu, damu itachukuliwa kwenye kidole.

Kwa nini mwanao?

Mwanao amechaguliwa kwa kuzingatia mbinu zakisayansi zitumiwazo kwenye utafiti.

Muda wautafiti:

Utafiti utafanywa kati ya mwezi wa januari hadi Julai 2017.

Kitakachofanyika kwa mwanao wakatiwa utafiti huu:

Mwanao atatarajiwa kutupa ujumbe kuhusu sehemu anayoishi na kabila, ujumbe huo utatumika kwa madhumuni ya utafiti pekee. Kisha atafanyiwa kipimo iliyotajwa mbeleni

Je,kuna madhara?

Hakunamadharayakuhusikakwenyeutafitihuu, mbalinauchungukiasiwakatiwakutoadamu, kilakitukitakuwasawa.

Je, kuna uzuriwa kuhusika kwenye utafiti?

Ndio, kujua hali yako ya sickle cell nimwanzo wakuzuia kuzaliwa kwa watoto ambao wako na ugonjwa wa sickle cell nakuendeleza jamii yenye afya bora.

Hakutakuwa na malipo kwa kuhusika kwenye utafiti.

Je, ukitaka kuuliza maswali zaidi?

Piga nambari ifuatayo: 0723810824 ambayo ni nambari ya mtafiti mkuu (Dkt. Einstein KIbet Watenga) napia 05333471-3008 ambayo ni namba ya tume kinacho toa kibali utafiti ufanyike,(IREC)

Je, ujumbe utakoyopeana utalindwa na mnagani?

Ujumbe wote utawekwa kwa usiri, Iwapo kutakuwa na umuhimu wakueleza mashirika mengine yanayokusudia mema kwa jamii, ujumbe huo utapeanwa lakini kwa namna ambayo inalinda haki zako .Kwa mfano tunaweza kuondoa jina na chochote kilekingine kinachoweza kukutambulishawa kati wakueleza mashirika mengine kuhusu matokeo ya utafiti.

SEHEMU YA 2: CHETI CHA KUKUBALI KUHUSIKA KWENYE UTAFITI.

Nimesoma pia nimeelezwa hadi nikaelewa yote ambayo hii utafiti inakusudia kufanya na nimekubali kwa hiari kujisa jili.

_____ _____ _____

Jina la Mshiriki Sahihi/Kidole Tarehe

_____ _____

Jina la Shahidi Uhusiano.Tarehe

_____ _____ _____

Jina la mwenye kuchukua kibali sahihi Tarehe

_____ _____ _____

Jina la Mtafiti, Sahihi Tarehe

Appendix IV: English Consent Form.

INFORMED CONSENT FORM FOR THE STUDY TITLED "PREVALENCE OF THE SICKLE CELL TRAIT AMONG ADOLESCENTS IN BUNGOMA COUNTY"

This Informed Consent Form has two parts:

• Information Sheet (to share information about the study with you)

• Certificate of Consent (for signatures if you choose to participate)

You will be given a copy of the signed Informed Consent Form

Part I: Information Sheet

Introduction:

You are being requested to allow your child to take part in this research study as a participant. This information is provided to inform you about the study. Please read this form carefully. You will be given a chance to ask questions. If you decide to let your child be in the study, you will be given a copy of this consent form for your records.

Taking part in this research study is voluntary. You are also free to have your child withdrawn from this study at any time. If after data collection you choose to quit, you can request that the information provided by you and your child be destroyed and thus would not be used in the research study. You have a right to information about the study or any issue of concern.

Information on Sickle cell disease:

Sickle cell disease is a genetic or hereditary blood disorder of the haemoglobin molecule which is part of the red blood cells that transport blood throughout the body of a human being. It is inherited in an autosomal recessive fashion. Any person carrying the sickle cell gene can transmit it to his or her offspring. Two categories of such persons exist:

- Those who have inherited the gene from one parent, and are referred to as sickle cell carriers or have the sickle cell trait. They lead normal lives and therefore do not show any signs of the disease and are unaware of they carry the gene.
- Those who have inherited the gene from both parents and referred to as having the sickle cell anaemia. They have lifelong signs of sickness which include anaemia (lack of blood), occlusion of blood vessels leading to painful episodes, stroke, chronic damage to various organs like the lungs, heart, joint and kidneys.

Purpose of the study:

The purpose of the study is to identify the persons with the sickle cell trait who may transmit sickle cell disease to their offspring without knowing and to provide health education so that they can be able to make informed choices to get spouses with normal haemoglobin so that their offspring can have normal haemoglobin or the trait.

Type of Research Project/Intervention:

The intervention is public health education on sickle cell disease and testing to identify the various haemoglobin variants among adolescents.

Why has your child been identified to Participate in this study?

Your child has been chosen for this study through a scientific process of selection that is used in research and also because he/she resides in Bungoma county.

How long will the study last?

The study will take a few minutes for the counseling and testing to be done. After the test and results have been explained to you and your child, the study will end. However, for more information you can call the principal investigator on 0723810824.

What will happen to your child during the study?

Your child will be asked to give us information about his ethnicity, gender and locality, we will then proceed to conduct the test mentioned above, we will report the results back to you and your child. This information will be confidential and stored securely to protect you and your child's privacy; it is meant for research purposes only.

What side effects or risks can I expect from being in the study?

There are no side effects except for the slight pain and discomfort when collecting the finger prick blood sample.

Are there benefits to taking part in the study?

Yes, Knowing ones status contributes to prevention of spread of sickle cell gene and disease by enabling one to be able to make an informed choice of spouse. Knowing the proportion of sickle cell trait carriers in the community will also help the government formulate policies to prevent spread of the gene and prevent birth of sickle cell disease children.

However no incentives will be offered for participating in the study.

Who do I call if I have questions about the study?

Questions about the study: Dr. Einstein Kibet 0723810824

Questions about your rights as a research subject: You may contact Institutional Review Ethics Committee (IREC) 053 33471 Ext.3008. IREC is a committee that reviews studies for safety and to protect the rights of study participants.

Will the information you provide be kept private?

All reasonable efforts will be made to keep your protected information (private and confidential. Protected Information is information that is, or has been, collected or maintained and can be linked back to you or your child. Using or sharing ("disclosure") of such information must follow National privacy guidelines. By signing the consent document for this study, you are giving permission ("authorization") for the uses and disclosures of your child's personal information. A decision to take part in this research means that you agree to let the research team use and share the child's Protected Information as described below.

As part of the study, Dr Einstein Kibet Watenga and his study team may share the results of your child's information e.g. laboratory tests, risk factors. These may be study or non-study related. They may also share portions of your child's medical record, with the groups named below:

- The National Bioethics. Committee,
- The Institutional Review and Ethics Committee,
- [Add others as appropriate, e.g., national institutes of health, representatives of {sponsor name},etc].

National privacy regulations may not apply to these groups; however, they have their own policies and guidelines to assure that all reasonable efforts will be made to keep your personal information private and confidential.

The sponsor may give your child's personal health information, not containing his/her name, to others or use it for research purposes other than those listed in this form. In handling your child's personal information, the sponsor, [PI] and associated staff will keep your child's information in strict confidence, and shall comply with any and all applicable laws regarding the confidentiality of such information.

The study results will be retained in your child's research record for at least six years after the study is completed. At that time, the research information not already in

your child's medical record will be deleted. Any research information entered into your child's medical record will be kept indefinitely.

Unless otherwise indicated, this permission to use or share your child's Personal Information does not have an expiration date. If you decide to withdraw your permission, we ask that you contact Dr Einstein Kibet Watenga, telephone 0723810824 in writing and let him know that you are withdrawing your permission. The mailing address is, email: einkibetz@gmail.com, P.o box 4606, 30100 Eldoret. At that time, we will stop further collection of any information about you. However, the health information collected before this withdrawal may continue to be used for the purposes of reporting and research quality.

You have the right to see and copy your child's personal information related to the research study for as long as the study doctor or research institution holds this information. However, to ensure the scientific quality of the research study, you will not be able to review some of your child's research information until after the research study has been completed.

Your child's treatment, payment or enrollment in any health plans or eligibility for benefits will not be affected if you decide not to take part. You will receive a copy of this form after it is signed.

Part II: Consent of Subject:

I have read or have had read to me the description of the research study. The investigator or his/her representative has explained the study to me and has answered all of the questions I have at this time. I have been told of the potential risks, discomforts and side effects as well as the possible benefits (if any) of the study. I freely volunteer to take part in this study.

_____	_____	_____
Name of Participant	Signature of subject/thumbprint	Date & Time
(Witness to print if the Subject is unable to write		
_____	_____	
Name of Representative/Witness	Relationship to Subject	
_____	_____	_____
Name of person Obtaining Consent	Signature of person Obtaining Consent	Date
_____	_____	
Printed name of Investigator	Signature of Investigator	Date

Appendix V: Assent Form.

ASSENT FORM FOR PARTICIPATION IN A STUDY TO DETERMINE THE PREVALENCE OF SICKLE CELL TRAIT AMONG ADOLESCENTS IN BUNGOMA COUNTY.

- The researcher has explained to me about the study.
- I understand that the researcher will ask questions about me.
- I understand that they will take a little blood from me.
- They need this sample so that they can determine my haemoblobin type and that my sickle cell status can be determined.

My parent/guardian has agreed that I should participate in the study, I also agree to the following. I voluntarily agree that I will participate as follows:

1. BE INTERVIEWED
2. FOR SAMPLES TO BE OBTAINED

Signature/mark of the child……

Name of child…….

Date……

Appendix VI: Swahili Assent Form.

RUHUSA YA KUHUSIKA KWAKO KWENYE UTAFITI WA KISAYANSI KUHUSU "SICKLE CELL TRAIT" KAUNTI YA BUNGOMA.

- Nimeelezwa kuhusu utafiti huu.
- Najua nitaulizwa maswali kunihusu.
- Nafahamu kwamba nitatolewa damu kiasi.
- Najua kwamba damu hiyo niyakubaini hali yangu ya sickle cell.

Mzazi wangu abamekubali nihusike kwenye utafiti huu ,mimi pia nimekubali kwa hiari kuhusika kwa namna ifuatayo:

1. KUHOJIWA.
2. KUCHUKULIWA DAMU

Sahihi/Alama ya mtoto……

Jina la mtoto…….

Tarehe……..

Appendix VII: The Counseling Process.

Pretesting counseling.

1. Welcome participant.
2. Reassure participant.
3. Ask what they know about sickle cell disease.
4. Go through basic information on sickle cell disease including transmission and severity of homozygous state.(sickle cell anaemia)
5. Emphasise on importance of screening and knowing status for prevention purposes.
6. Discuss implication of results.
7. Explain to participant how the rapid immunoassay using sickle scan test works and how results are read.
8. Get informed consent from participant.
9. Collect demographic data on questionnaire.
10. Test participant.(see appendix V on how to test)

Post testing counseling.

1. Reassure participant.
2. Go through the results together.
3. Discuss implications of result obtained.
4. Offer medical advice to participants.

Appendix VIII: The Sickle Scan™ Test Protocol.

STEP 1.

Do not open pouch until ready to use.

Prepare necessary materials:

A: Sickle SCAN™ cartridge

B: PreTreatment Buffer Module

C: Capillary Sampler (5 µL volume)

*Label PreTreatment Module and Test cartridge with patient ID.

STEP 2.

Obtain a fingerstick specimen using standard laboratory protocols.

*For heelstick or intravenous sampling follow standard laboratory protocols.

STEP 3.

Using Capillary Sampler, obtain 5 µL of fingerprick blood specimen.

*Take care to draw sample by capillary action; do not squeeze dispensing bulb.

STEP 4.

Open PreTreatment Module and immerse the Sampler tip into the Module. Dispense the specimen into the buffer.

*Take care in opening the PreTreatment Module, as it contains a premeasured volume of extraction buffer.

STEP 5.

Replace and tightly screw the two-piece cap onto the Module.

STEP 6.

Invert the module and mix 3 times, allowing complete treatment of the specimen with buffer.

STEP 7.

Remove colored cap of the PreTreatment Module.

*Dispense 3 drops into waste to allow removal of any air bubbles in the dropper.

Immediately dispense 5 drops into the Sickle SCAN™ cartridge.

*Test on a level surface at room

STEP 8.

Allow test to run for 5 minutes.

*Test results that have run over 24 hours are invalid.

STEP 9.

Read the results of the Sickle SCAN™ by viewing the detection window.

POSSIBLE RESULTS.

Appendix IX: sickle scan™ validation certificate.

FORM B

S. No.000014

REPUBLIC OF KENYA
Ministry of Health

CERTIFICATE OF VALIDATION FOR REAGENTS AND EQUIPMENT
(CAP 253A & LEGAL NOTICE NO. 113 OF 2013)

IT IS HEREBY CERTIFIED THAT THE EQUIPMENT AND/OR REAGENT / INVITRO DIAGNOSTIC DEVICE AS DESCRIBED HERE UNDER HAS BEEN VALIDATED SUBJECT TO THE CONDITIONS INDICATED:-

APPROVED NAME
Sickle Scan BM-SCS-PI-006.P

TRADE NAME UNDER WHICH MARKETED
Sickle Scan Lateral Flow Catridge

VALIDATION NUMBER
KMLTTB / POC / 000014 / 2016

ACTIVE INGREDIENTS AND QUANTITIES PER UNIT
Monoclonal anti-hemoglobin, polyclonal anti-HbA & HbS

METHOD OF EXPERIMENT TO ESTIMATE INACCURACY/BIAS
Qualitative Lateral Flow Immuno Assay - Sens 98% : Specificity 99%

DETECTION LIMIT OF EXPERIMENT & ESTIMATION OF REFERENCE/REPORTABLE RANGE
HbA 40% & HbS 1%

REPLICATION & INTERFERENCE EXPERIMENT
Stability Assays not included

FORM OF PREPARATION
Catridge

CONDITIONS UNDER WHICH EQUIPMENT AND/OR REAGENT IS VALIDATED
Laboratory Assay

NAME & BUSINESS ADDRESS OF MANUFACTURER / VENDOR / SUPPLIER / DISTRIBUTOR
BioMedomics, Inc., 6 Davis Drive, Durham, NC 27709, USA

REGISTERED BUSINESS NAME & ADDRESS
Medomix Concept Limited, Uchumi House, Aga Khan Walk, Nairobi, Kenya.

15th April 2016	15th April 2019
DATE OF VALIDATION	EXPIRY
REGISTRAR	CHAIRMAN

Appendix X: certificate of registration for medomix concept ltd.

REPUBLIC OF KENYA
MINISTRY OF HEALTH

KENYA MEDICAL LABORATORY
TECHNICIANS & TECHNOLOGIST BOARD

THE KENYA MEDICAL LABORATORY TECHNICIAN AND TECHNOLOGISTS BOARD

(MLTI Act No.10 of 1999) cap 253A

Certificate Of Registration

Serial No. **0113**

of manufacturers and other Related parties Dealing with Medical Laboratory Equipments and reagents (IVDs).

This is to certify that

MEDOMIX CONCEPT LTD

Situated at

UCHUMI HSE 10TH FLR AGA KHAN WALK, NAIROBI

Pursuant to section 25 of the MLTT Act 1999, with amendments on Legal notice No 113/2011. The above Mentioned Company is registered and authorized to distribute/import/supply medical laboratory equipment and reagents in conformity with provisions of the Act.

This certificate is issued under the seal of the board

V0113

Vendor Reg.No

Registrar

17/11/2015

Date of issue

airman

DISCLAIMER;

This is not a certificate for validation ... *al and reagents. See overleaf*

Appendix XI: IREC Approval

INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC)

MOI TEACHING AND REFERRAL HOSPITAL
P.O. BOX 3
ELDORET
Tel: 33471/2/3

MOI UNIVERSITY
SCHOOL OF MEDICINE
P.O. BOX 4606
ELDORET

Reference: IREC/2016/151
Approval Number: 0001742

26th September, 2016

Dr. Einstein Kibet Watenga,
Moi University,
School of Medicine,
P.O. Box 4606-30100,
ELDORET-KENYA.

INSTITUTIONAL RESEARCH & ETHICS COMMITTEE
26 SEP 2016
APPROVED
P. O. Box 4606-30100 ELDORET

Dear Dr. Watenga,

RE: FORMAL APPROVAL

The Institutional Research and Ethics Committee has reviewed your research proposal titled:-

"The Prevalence of Sickle Cell Trait among Adolescents in Bungoma County, Kenya".

Your proposal has been granted a Formal Approval Number: ***FAN: IREC 1742*** on 26th September, 2016. You are therefore permitted to begin your investigations.

Note that this approval is for 1 year; it will thus expire on 25th September, 2017. If it is necessary to continue with this research beyond the expiry date, a request for continuation should be made in writing to IREC Secretariat two months prior to the expiry date.

You are required to submit progress report(s) regularly as dictated by your proposal. Furthermore, you must notify the Committee of any proposal change (s) or amendment (s), serious or unexpected outcomes related to the conduct of the study, or study termination for any reason. The Committee expects to receive a final report at the end of the study.

Sincerely,

PROF. E. WERE
CHAIRMAN
INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE

cc	CEO	-	MTRH	Dean	-	SOP	Dean	-	SOM
	Principal	-	CHS	Dean	-	SON	Dean	-	SOD