MARDEA F. ZAWAY

THE PREVALENCE OF SICKLE CELL TRAIT IN ZIMBABWEANS STUDENTS IN NORTHERN CYPRUS

MSc. THESIS

December, 2024

NEAR EAST UNIVERSITY

INSTITUTE OF GRADUATE STUDIES

DEPARTMENT OF MEDICAL GENETICS

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Approval

We certify that we have read the thesis submitted by MARDEA F ZAWAY titled **THE PREVALENCE OF SICKLE CELL TRAIT IN ZIMBABWEANS IN STUDENTS IN NORTHERN CYPRUS"** and that in our combined opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Sciences.

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DECLARATION

I hereby declare that all information documents analysis in this thesis titled "**The Prevalence of Sickle Trait Among Zimbabweans Students in Northern Cyprus**"has been collected and presented according to the academic rules and ethical guidelines of Institute of Graduate School, Near East University. I also declare that as required by these rules and conduct,I have fully cited and reference information and data that are not original to this study.

MARDEA F. ZAWAY

DEDICATION

With a cheerful heart, I hereby dedicate this astonishing thesis to my husband, Mr. John K. Weetor, and my Handsome babies Marci-Joe Heaven Peanut Weetor and Edward A-ron Choice Sunshine Weetor**.**

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Who else could it be if it’s not all God? God is my only way-maker. This thesis is a reality with the help, support, and hardworking of my supervisor Assoc Prof. Dr. Mahmut C. Ergoren and mostly importantly, Prof. Dr. Pinar Tulay her guidance, innovation ,persistence's and over all mentorship during my academic journey. Thank you very much to Medical Biology and Genetics department, for all they've done to welcome Africans, and Liberians in particular, to the Near East University community.

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MARDEA F. ZAWAY

Abstract

The Prevalence of Sickle Trait in Zimbabweans in Northern Cyprus

MARDEA F. ZAWAY

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Background: This thesis investigates the Prevalence of Sickle Cell Disease among Zimbabweans Students in Northern Cyprus. Sickle cell disease (SCD) is a hereditary disorder caused by abnormalities in the HBB gene, which codes for the hemoglobin component. Every year, between 300,000 and 400,000 babies are thought to be afflicted by the illness, with Sub-Saharan Africa accounting for the vast majority of cases.

Method: Blood samples from 108 randomly chosen Zimbabwean students were taken with a glass syringe and put in 2.5-ml Ethylenediaminetetraacetic acid (EDTA) tubes.These samples were used for the extraction of genomic DNA, which was then processed in class II laminar flow hood using the appropriate pipettes. During the process of extraction, this step was taken so that there would be as little chance of contamination as possible. Every one of the several DNA synthesis solutions went through an ultraviolet (UV) treatment so that any tainted DNA could be rendered inert, the replication of the DNA could be stopped, and the DNA's deterioration could be avoided. In order to extract and purify genomic DNA from whole blood in accordance with the procedure that was outlined by the business that manufactures DNA Isolation Kit (Hibrigen Biotechnology LTD, Gebze, Turkey) was used. The PCR process using the three main PCR amplification techniques over the course of 35 cycles were used to amplified the DNA.

The Hardy-Weinberg equilibrium (HWE) was calculated using the Pearson Chi-Square (2) test to estimate genotype distributions and allele frequencies. A p-value of less than 0.05 is considered statistically significant. The exact HWE test was performed using the website https://www.cog-genomics.org/software/stats, and the results were interpreted using the GraphPad Prism application (GraphPad Software, Inc., San Diego, California, United States).

Result: The PCR genotyping results indicated that no individual exhibited heterozygosity, while twenty-seven percent of the 108 samples (AS, 0.27%) had the features for sickle

cell diseases among Zimbabwean students studying in Northern Cyprus. The A allele frequency was found to be 0.87% among the Zimbabwean students studied in the Northern Cyprus population, whereas the S allele frequency was calculated to be 0.12%. The gene-counting method was used to calculate genotype distributions and allele frequencies, and their conformance to the Hardy-Weinberg equilibrium was determined using the goodness-of-fit 2 test, with a P value of less than 0.05 indicating significant disequilibrium. The Hardy-Weinberg equilibrium (P = 0.332) was seen in the genotype distributions of the *HBB*) gene.

Conclusion: This research suggests that the prevalence of sickle cell disease in Zimbabwe is low in comparison to the prevalence in many other nations in Africa and throughout the world. Multiple studies have shown that African countries, particularly those in sub-Saharan Africa, have high rates of SCD. Between 50 and 60 percent of newborns in Sudan are diagnosed with SCD each year.

Keywords*:* Sickle Cell Disease, Hemoglobin, Hereditary, Genotype, Abnormalities

Özet

Kuzey Kıbrıs'taki Zimbabvelilerde Oraklık Özelliğinin Yaygınlığı

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Yüksek LisansTıbbi BiyolojiveGenetik

Aralık 2024 70 Sayfa

Arka Plan: Bu Tez, Kuzey Kıbrıs'taki Zimbabveliler arasında Orak Hücre Hastalığının Yaygınlığını araştırmaktadır. Orak hücre hastalığı (SCD), hemoglobin bileşenini kodlayan HBB genindeki anormalliklerin neden olduğu kalıtsal bir hastalıktır. Her yıl 300.000 ila 400.000 arasında bebeğin hastalıktan etkilendiği düşünülüyor ve vakaların büyük çoğunluğunu Sahra Altı Afrika oluşturuyor.

Yöntem: Rastgele seçilen 108 Zimbabveli öğrenciden kan örnekleri cam şırıngayl aalınarak 2,5ml'lik Etilen diamintetra asetikasit (EDTA) tüplerine konuldu. Bu örnekler genomic DNA'nın ekstra ksiyonu için kullanıldı ve daha sonra sınıf II laminerakışlıka portada işlendi. Uygun pipetleri kullanın. Ekstraksiyon işlemi sırasında bu adım, kirlenme olasılığının mümkün olduğu kadar azolması için atıldı. Çeşitli DNA sentezi çözeltilerinin her biri bir ultraviyole (UV) işleminden geçirildi, böyle herhangi bir kusurlu DNA etkisiz hale getirilebildi, DNA'nın kopyalanması durdurulabil dive DNA'nın bozulması önlenebildi. Tam Kandan genomic DNA'nın DNA İzolasyon Kiti üreten işletmenin (Hibrigen Biyoteknoloji LTD, Gebze, Türkiye) belirttiği prosedüre uygun olarak ekstra kteedilmesi ve saflaştırılması için kullanıldı. DNA'yı amplifiye etmek için 35 döngü boyunca üç ana PCR amplifikasyon tekniğini kullanan PCR işlemi kullanıldı.

Hardy-Weinberg dengesi (HWE), genotip dağılımlarını ve alel frekanslarını tahmin etmek için Pearson Ki-Kare (2) testi kullanılarak hesaplandı. 0,05'ten küçük bir p değeri istatistiksel olarak anlamlı kabul edilir. Tam HWE testi https://www.cog-genomics.org/software/stats web sitesi kullanılarak gerçekleştirildi ve sonuçlar GraphPad Prism uygulaması (GraphPad Software, Inc., San Diego, California, Amerika Birleşik Devletleri) kullanılarak yorumlandı.

Sonuç: PCR genotipleme sonuçları hiçbir bireyin heterozigotluk göstermediğini gösterirken, Kuzey Kıbrıs'ta öğrenim gören Zimbabveli öğrenciler arasında 108 örneğin

yüzde yirmiyedisinin (AS, %0,27) orak hücre hastalığı özelliklerine sahip olduğu görüldü. Kuzey Kıbrıs nüfusunda öğrenim gören Zimbabveli öğrencilerde A allelfrekansı %0,87, S allelfrekansıise %0,12 olarak hesaplandı. Genotip dağılımlarını ve alelfrekanslarını hesaplamak için gen sayma yöntemi kullanıldı ve bunların Hardy-Weinberg dengesine uygunluğu, uyum iyiliği 2 testi kullanılarak belirlendi; 0,05'ten düşükbir P değeri, önemli dengesizliği gösterir. HBB geminin genotip dağılımlarında Hardy-Weinberg dengesi (P=0.332) görüldü.

Sonuç: Bu araştırma, Zimbabwe'de orak hücre hastalığı prevalansının, Afrika'daki ve dünyadaki diğer birçok ülkedeki prevalansakıyasla düşük olduğunu göstermektedir. Çok sayıda çalışma, Afrika ülkelerinin, özellikle de Sahra altı Afrika'dakilerin, yüksek AKÖ oranlarına sahip olduğunu göstermiştir.Sudan'da her yıl yeni doğanların yüzde 50 ila 60'ına AKÖ tanısı konuyor.

AnahtarKelimeler:OrakHücreHastalığı, Hemoglobin, Kalıtsal, Genotip, Anormallikler

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List of Abbreviations

AS: Sickle Cell Carrier (Heterozygous)

Bp: Base Pair

CBC: Complete Blood Count

CDC: Center for Disease Control

Dbsnp: Single Nucleotide Polymorphism Database

DNA: Deoxyribonucleic Acid

EDTA: Ethylenediaminetetraacetic

Glu: Glutamic Acid

HB: Human Hemoglobin

***HBB*:** Human Subunit Beta Gene

HBs: Embryonic Hemoglobin

HBSC: Hemoglobin Sickle Cell

HBF: Fetal Hemoglobin

HbSM: Hemoglobin Sickle Mutation

HbSWT: Hemoglobin Sickle Wide Type

HbSP: Hemoglobin Sickle Probe

HWE: Hardy-Weinberg equilibrium

MM: Master Mix

PCR: Polymerase Chain Reaction

RBCs: Red Blood Cells

RFL: Restriction Fragment Length Polymorphism

SCA: Sickle Cell Anemia

SCD: Sickle Cell Disease

SCT: Sickle Cell Trait

SNPs: Single Nucleotide Polymorphism

SNVs: Single Nucleotide Variants

SS: Sickle Cell (Homozygous)

UV: Ultraviolet

CHAPTER I

Introduction

Sickle cell Disease

Genetic diseases like sickle cell disease (SCD) are thought to be caused by changes in the *HBB*gene, which creates the hemoglobin subunit, according to (Kato et al., 2018). It is believed that between 300,000 and 400,000 newborns are affected by the disease every year around the world, with the majority of cases occurring in sub-Saharan Africa. A group of sickle globin molecules can stick together. Erythrocytes that mostly have sickle hemoglobin polymers on them look like they have wavy lines and are more likely to break apart. Additional pathophysiological processes, such as immune system activation and vasoconstriction, can alter the SCD phenotype. The phenotypic multifaceted nature of SCD, a characteristic of the disorder, sets it apart from other forms of the disease. Some examples of frequent acute effects are acute pain episodes, acute chest syndrome, and stroke (Kato et al., 2018).

Chronic problems, such as chronic kidney disease, have the potential to damage all of the body's organs at some point. It is possible to lessen the severity of the condition using hydroxycarbamide, blood transfusions, and hemopoietic stem cell transplantation. Early diagnosis improves a patient's chances of survival. Some nations have developed universal newborn screening programs, although this may be difficult in contexts with low income and a high disease burden. Sickle cell disease, usually referred to as SCD, is a multi-system disorder that affects a disproportionately high number of African Americans in the United States. It is also the most common inherited illness in certain parts of the world. Around 300,000 infants in the United States receive a diagnosis of sickle cell anemia every year. A mutation causes this condition, passing down through families in an autosomal recessive manner. There is still a lack of complete understanding of the phenotypic manifestation of the illness. Environmental factors like temperature, air quality, infections, fetal hemoglobin, and genetic variants affect the condition's presentation. The clinical symptoms are diverse and may affect many systems; they also often result in a decreased life expectancy, according to studies conducted by (Galadanci et al., 2019) and (Moody, K. et al., 2019).

Sickle cell disease is a prevalent, severe, single-gene disease present worldwide. This condition, which can affect multiple body systems and is characterized by bouts of acute illness and the gradual loss of organ function, is prevalent. Ever since the initial discovery in 1910 of erythrocytes with a distinctive sickle shape, there has been continuous improvement in our understanding of the condition (Herrick, 1910). In 1949, Paulin discovered electrophoretic aberrations in sickle hemoglobin (HbS). To describe the condition, they coined the term "molecular sickness." Many research studies on the genetics and hemoglobin biophysics that cause the disease have helped us learn more about other molecular problems. It is not clear if hydroxycarbamide and blood donations can help with some conditions, and no medicines have been found yet that directly target the pathophysiology of this illness. As a direct result of this, palliative care constitutes the bulk of the treatment for sickle cell disease. Since the beginning of this century, residents of Africa have become aware of a sickness that is characterized by severe symptoms and has a very high fatality rate. The first person to discover sickle cell disease is James Herrick, a medical practitioner and researcher for Chaucer. Grenada's medical student diagnosed the patient with sickle cell disease. Numerous discoveries during the first decade of the 20th century paved the way for further investigation into sickle cell disease. Linus Pauling first demonstrated the abnormal electrophoretic mobility of hemoglobin (Pauling et al., 1949).

Vernon Ingram discovered the sickle cell disease defect as a single amino acid change in the sickle cell protein complex (HbS). Max Perutz elucidated the structure of hemoglobin, and Janet Watson explained the molecular basis of its function. The protein known as hemoglobin is responsible for carrying oxygen across the body. Since well over a century ago, people in Africa have been aware of the existence of a sickness that is characterized by excruciating episodes and results in an untimely death. Onwubalili (1983), a student of medicine from Grenada, and James Herrick (1910), a medical doctor and Chaucer scholar from the United Kingdom, are credited with making the first discovery of sickle cells. The first fifty years of the twentieth century saw several significant discoveries in sickle cell disease. These discoveries are just a few examples: Linus Pauling et al. (2004) demonstrated abnormal hemoglobin movement on an electrophoretic gradient in a patient with the condition. It was discovered by Vernon

Ingram in 1957 that the fundamental reason for the illness was substituting one amino acid for another in the hemoglobin molecule of sickle cells (HbS). (Perutz et al. 1960), who decoded hemoglobin's structure, described its role's molecular underpinning and observed that symptoms manifested themselves.

Hemoglobin

A blood condition known as sickle cell disease (SCD) is inherited and characterized by anomalies in hemoglobin (Creary et al. 2007). Red blood cells may become sickled or destroyed when sickle cell disease occurs. This is because the condition lowers the oxygen-carrying capacity of hemoglobin (Barakat et al. 2008). This ailment manifests quite differently in individuals and may affect any part of the human body (Bloom, 1995). Dr James Herrick, a physician from Chicago, was the first American to record and recognize extended, sickle-shaped hemoglobin in 1910 accurately. This hemoglobin was discovered in a blood smear obtained from an anemic student from Grenada. Herrick was the first American to do so. According to Ogamdi (1994), most credit for popularizing "sickle cell disease" goes to Herrick. Herrick's sickle-shaped red blood cells were the source of a multitude of difficulties, including his short height, delayed puberty, and chronic anemia. He also suffered from vaso-occlusive pain episodes, ischemic organ damage, infections, and vaso-occlusive pain episodes (Barakat et al. 2008). Since many millennia ago, Africa has been home to a sizable population suffering from sickle cell disease. Reports indicate that sickle cell disease was a common condition in West Africa long before it was recognized as such in the United States. The people of West Africa reportedly referred to it by several other names before its discovery in the United (Reid & Rodgers, 2007).

Hemoglobin, from the Greek words for blood (haem) and protein (globin), is found in red blood cells and circulates throughout the body. It is essential for carrying oxygen from the lungs to the rest of the body's tissues (Perutz, 1970). Because of how its protein structure is organized, hemoglobin forms a strong bond with oxygen. The alpha chain of this globin molecule has a total of 144 amino acids, whereas the beta chain has a total of 146 amino acids (Kendrew, 1961). Hemoglobin comprises several structures, including the first, second, third, and fourth. The "primary structure" of hemoglobin is so named because it

comprises the structures of the amino acids that make it up. Each alpha and beta chain folds into an alpha-helical shape linked by hydrogen bonds to form the secondary structure. This is the initial stage of building the secondary structure (Perutz, 1970). Once the helical formations fold in on them to create globular structures, this marks the completion of the final step in creating the tertiary structure. These helices link together to create a pouch home to the heme, which is responsible for giving blood its distinctive red color. Two alpha chains and two beta chains, for a total of four polypeptide chains, are linked together to make the quaternary structure of a complete hemoglobin molecule (Pauling et al., 1949).

Because of a change in the sequence of nucleotides in the DNA that codes for hemoglobin, sickle hemoglobin has a different main structure than normal hemoglobin. This mutation does not affect normal hemoglobin. This mutation causes a point mutation in the amino acid sequence at the sixth position. As a result, glutamic acid 6 in S hemoglobin is converted to valine, while glutamic acid 6 in C hemoglobin is changed to histidine (Ingram, 1957). Because of its typical stability, normal hemoglobin does not exhibit this mutation. So, even though valine is known for its hydrophobic tendency, glutamic acid is recognized for its hydrophilic nature. As oxygen concentrations drop, globin folding patterns alter. This is due to valine, a component of hemoglobin, which allows the molecules to reject water while still attracting one another (Bunn, 1997). The red blood cells take on the appearance of a sickle due to this distortion in their standard shape, a tool farmers utilize. Valine is a hydrophobic amino acid. This sickled structure promotes vascular inflammation and other alterations that limit blood flow. It also plugs capillaries, which inhibit red blood cells from providing oxygen and stop red blood cells from delivering oxygen (Rees et al., 2010).

Hemoglobin's Amino Acid Sequence in its

Normal HB Form - valine – histidine - leusine – threonine – proline –GLUTAMIC ACID -Glutamic acid-lysine

HB S- valine – histidine – leusine – threonine – proline –VALINE- glutamic acid -lysine

HB C- valine – histidine – leusine – threonine – proline –HISTIDINE –glutamic acid lysine

Occupies the sixth position amino acids.

The interplay of at least two genes, one of which originates from both parents, determines the quantity of hemoglobin that is present in a person's blood. The presence of a pair of copies of the sickle hemoglobin gene, one inherited from each parent, is what leads to sickle cell disease (Serjeant, 1992). As a result, the illness manifests itself in sickle cells (homozygous recessive). It is possible for a person to receive one sickle gene and one normal gene from each parent, which is known as being "heterozygous" for sickle cell disease. This is what is meant by the phrase "sickle cell characteristics." Those who have sickle cell characteristics may be healthy and have ordinary lives, but they are more susceptible to develop sickle cell disease symptoms if they are exposed to oxygen deprivation, such as at high altitude. They are also known as sickle cell gene carriers and sickle cell trait carriers (CDC, 2020).

Hemoglobin Variants

People with sickle cell anemia, which is a severe form of inherited hemoglobin that causes red blood cells to be shaped in a way that looks like crescents when oxygen levels are low, have a different type of hemoglobin called hemoglobin S (Rees, et al., 2010). The sickle-shaped abnormal cells may lodge in the blood's microcirculation before they prematurely die. This may cause damage to the tissue. Sickle cell disease is more prevalent among people of African origin, even though Middle Easterners may also suffer from the condition (Serjeant, 1992). The genes that code for the 1–2, 2–, 3–, 4–, 5–, G–, A–, and 6– globin chain variants have gone through many different mutations, which lets them be put into many different groups. Most changes to Hb result from a single base change in the genetic components of these proteins. This is basically what all of them are (almost 100%). Around 6% of all variants are considered to be abnormal. When hemoglobin is made, it can change the C- or N-terminal of the hybrid globin, cancel or add globins, and do binary substitution on the exact globin string. There are over a thousand variations of hemoglobin found in humans, each with a different level of

anatomical implications. People are known to harbor these variants (Bunn & Forget, 1986).

Hemoglobin Biochemistry and Pathophysiology

The Mutations that change the fundamental structure of globin, referred to as hemoglobinopathies, or decrease the expression of globin genes, referred to as thalassemia, are the two types of mutations that cause hemoglobin diseases (Weatherall, 2001). On rare occasions, a single mutation may affect both the structure of the gene and the degree to which it is expressed. These disorders are collectively referred to as thalassemichemoglobinopathies. Although various hemoglobinopathies and thalassemia might result in comparable clinical symptoms and pathophysiological implications at the level of the defective erythrocyte, such generalizations do not apply to every hemoglobin disease. For example, only sickle hemoglobin (HbS) polymerizes, which is what causes sickle cell disease and its many symptoms (Rees et al., 2010).

On the other hand, thalassemia major is characterized by highly inefficient erythropoiesis. The following four chapters will focus on discussing the implications that abnormalities in the hemoglobin have for the erythrocyte membrane, the biology of nitric oxide, and the longevity of red blood cells. A chapter that serves as the conclusion provides an update on the animal models that make it possible to investigate this pathobiology in specific detail. All of these chapters were written specifically for this updated book version. Both sickle cell disease and thalassemia require adjustments to be made to the vascular tone and flow in order to compensate for the chronic hypoxia and hemolysis (Steinberg, 1999). In sickle cell disease, red cells' deformability depends on the oxygen tension within the intravascular space. This is because hypoxia leads to HbS polymerization, which causes erythrocyte sickling and Vaso occlusion. Damage caused by reperfusion in sickle cell disease is characterized by excessive oxidant production, endothelial activation and dysfunction, and inflammation. This damage is brought on by Vaso occlusive events (Hebbel, 2011). The comparable red cell defects in thalassemia lead to hemolysis and decreased red cell deformability, both of which support the pathobiology of the vascular system. Both oxidative stress and the precipitation of an abnormally high number of globin chains are responsible for these abnormalities (Fibach & Rachmilewitz, 2008).

There are about one hundred thousand persons in the United States who currently have sickle cell disease alone, in addition to millions more in other countries around the globe. It is possible for sickle cell disorder, a genetic ailment passed down across families in an autosomal-recessive way and may be passed down over a long time, to be passed down from generation to generation. According to the results of the Global Effect of Disease Survey, there are 3.2 million people who have sickle cell disease, 43 million people possess the trait of sickle cell (are variant carriers), and 176,000 people pass away each year as an immediate result of challenges associated with SCD (Piel et al., 2013). It is a catch-all word that refers to several genetic mutations in the beta-globin gene that all lead to the same clinical illness. These variations are grouped under one umbrella term. Sickle cell illnesses refer to genetic alterations affecting a person's red blood cells. Seventy percent of patients with sickle cell disease have African ancestry, and the most prevalent type of sickle cell illness is sickle cell anemia (which, for the benefit of those interested in this review, will be described together with other sickle cell diseases). The presence of two copies of the beta-S (S) allele on chromosome 11p15.5 is required for the development of sickle cell anemia since this is the requirement for the disorder to manifest itself (Ingram , 1957). This is due to a change in the n-linked gene's 6th codon, which shifts from GAG to GTG at the point in question. DbSNP Rs334 (T, T) is the name given to this particular SNP (1, 3, 5, 6). Patients with sickle cell anemia have an altered hemoglobin tetramer HbS (2s2) in their erythrocytes as a result of the replacement of a hydrophobic single amino acid residue for a hydrophilic glutamic acid residue (Glu) at location 6, also on the hemoglobin chain (Ingram , 1957). This results in the sickle cell anemia patient's erythrocytes having a sickle shape. These patients also have a higher risk of developing sickle cell crises than the general population does. Other types of sickle cell disease are caused by the S mutation being passed down either homozygously (HbSS) or in combination with other mutations (HbSC, HbSD, HbSO/Arab, HbSE, or a -thalassemia allele; HbS/-thal0 or HbS/-thal+). Homozygous transmission of the S mutation is referred to as sickle cell trait. The following sections of this article discuss these mutations and the molecular and cellular mechanisms connected to them (Serjeant, 2013).

Hemoglobinopathies

The most common disorders of recessive monogenic inheritance worldwide are known as hemoglobinopathies (Weatherall, 2001).Mutations that alter the structure of globin chains cause thalassemia and other abnormal or variant forms of hemoglobin. These mutations also hinder the development of the globin chains, which are essential to the formation of hemoglobin. Usually, these mutations pass down through generations in an autosomal recessive manner. These conditions cause phenotypes that are clinically significant and vary in severity. These can be homozygous or genetic compound states (for example, thalassemia major, intermedia, sickle cell syndromes, and HbE syndromes). While heterozygotes may not display any symptoms, they display a wide range of hematological characteristics that are often useful in identifying them (Steinberg, 199). There are many types of thalassemia and many different hemoglobin genotypes that can interact with each other when they are co-inherited. This can cause complicated hematological symptoms that are hard to understand and need more research in family studies and DNA analysis to determine the correct diagnosis (Weatherall, 2010).

Changes in the beta-globin gene cause hemoglobinopathies, a group of genetic diseases (LL & Clegg, 2001). Researchers can diagnose these conditions by using characteristic and analytical changes in the hemoglobin production process. Tropical regions are widespread with hemoglobinopathies, where researchers have identified nearly a thousand different mutations in a single gene (Hardison et al., 2002). Because these blood disorders are caused by groups of mutations that mess up the structure or production of globin genes, they usually make it hard for hemoglobin to work.

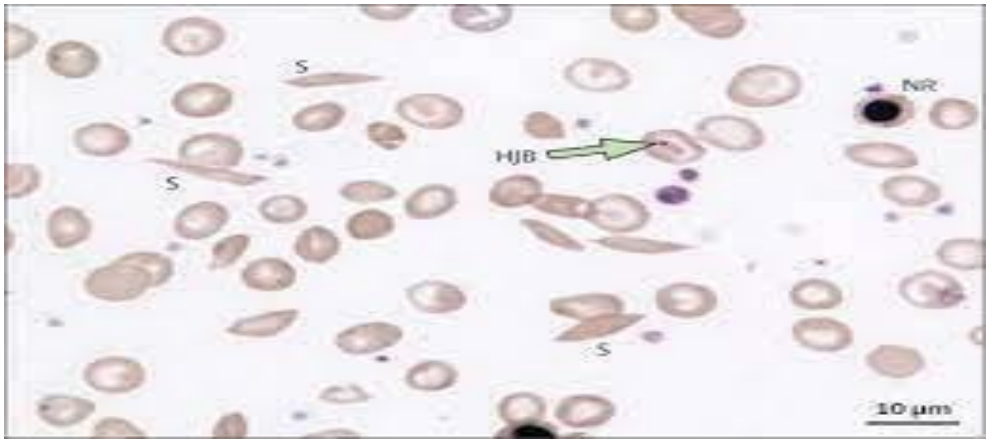
Classification

It is common to hear the term "sickle cell sickness," which means homozygosity with the S gene. However, the term "sickle-cell disease" often refers to all other genotypes that cause the typical medical picture (Steinberg, 1999). This is because sickle cell anemia is the only kind of sickle cell illness that affects red blood cells. Sickle cell anemia is among the most prevalent signs of sickle cell disease. Most people with sickle cell illness also have hemoglobin SC sickness, often known as HbSC disease. The homology of the ßSand ßC alleles causes this disease (Rees et al., 2010). People of African ethnicity are

far more likely to develop sickle cell anemia, a kind of sickle cell disease that occurs often. It accounts for about 70% of all cases of sickle cell disease. The S and -thalassemia alleles are handed down from parents to children. This generates HbS/-thalassemia, the third most frequent form of sickle cell disease. This combination creates the sickness. The kind of thalassemia mutation present dictates the extent to which this disease manifests itself. In addition to the many kinds of HbS and thalassemia, eight other genotypes that cause sickle cell disease have been identified; nevertheless, the bulk of these sickle cell disorders are exceedingly uncommon (Piel et al., 2013).

Figure 1.

A peripheral blood smear of a person with sickle- cell anemia.



This blood film shows cells that are permanently crinkled (marked with an "S"), red blood cells with nuclei (marked with an "NR"), and Howell-Jolly bodies (marked with an "HJB"), which are most often caused by a lack of spleen (marked with an "HJB").
Source;(Rees et al 2010).

Etiology and Epidemiology

The mutation in the beta chain of hemoglobin leads to sickle cell anemia in affected individuals. The mutation occurs at position six on the chromosome, replacing glutamic acid with valine (Ingram, 1957). Hemoglobin is an essential protein, and red blood cells store a significant amount of this molecule. Hemoglobin is also known by the acronym RBC, which stands for red blood cell iron. It is composed of four distinct globin chains, two of which are derived from alpha-globin, located on chromosome 16, and the

other two are derived from beta-globin, located on chromosome 11 (Steinberg, 1999). Sickle cell anemia, commonly called sickle cell disease, may be traced back to a defect in the beta-globin chain as the primary underlying cause. This mutation causes the sixth position in the chain to change from a positively charged glutamic acid to an uncharged valine. Mendelian genetics principles transmit the mutation from generation to generation. More specifically, the mutation is transmitted in an autosomal dominant manner (Piel et al., 2013). One particular mutation is responsible for the most serious form of SCD, also called HBSS disease or SCA, and occurs in both individual copies of their DNA. This causes the illness to manifest in its most severe form. The phenotypic traits of the sickness known as HBS-Beta-0 are analogous to those of the HBSS illness. The sickle cell mutation and the beta-naught variant of thalassemia have a role in developing this disease. Only in a heterozygous manner can one inherit HbAS (Steinberg, 2001).

People with HbAS are not considered part of the SCD spectrum because they rarely exhibit SCA-specific symptoms. This is the reason why HbAS people are not considered to be part of the SCD spectrum. This is due to the hereditary nature of the HbAS disease. Screening measures, blood donations, or delivery are likely the only ways to detect them. The person also has a lot of different types of composite heterozygotes, which happen when they inherit one copy of the mutant beta-globin gene along with one copy of another gene that is broken. This is the second most common sickle cell disease (SCD) type. It happens when one copy of the gene that causes sickle cell disease lives with a copy of the mutant hemoglobin C gene. This combination results in a sickle cell trait that causes red blood cells to become sickle-shaped. The affected individual inherits the sickle cell trait due to this combination. This combination results in sickle cells that cannot properly carry oxygen throughout the body. Lysine is substituted for the glutamic acid that is ordinarily found in position 6 of the beta-globin chain during the hemoglobin (HbC) manufacturing process. HbSC illness affects one out of every three people in the United States (Platt, 2008).

A limited number of epidemiological investigations have been conducted about sickle cell disease. Sub-Saharan Africa is widely known for having a higher prevalence of sickle cell disease (SCD) and HbA1c than other regions. We hypothesize this is due to the innate resistance of people with HbAS to severe forms of Plasmodium falciparum malaria.

It is projected that in the area of sub-Saharan Africa in 2010, a total of over 3.5 million newborns are expected to be born with HbAS, as well as more than 230,000 infants born with SCA. These numbers are based on estimates made by researchers. Researchers estimate that 75 percent of all SCD-related births occur in Sub-Saharan Africa. Patients suffering from HbSC-related disorders make up the vast majority of the population in West African countries. Statistics released by the Centers for Disease Control and Prevention (CDC) in the United States indicate that the number of people with sickle cell disease (SCD) surpasses 100,000. According to data provided by the Centers for Disease Control and Prevention (CDC), sickle cell disease (SCD) affects one in 365 people of African-American descent and one in thirteen children whose parents are both of African-American descent. Furthermore, sickle cell disease affects the blood of one in every 365 newborns delivered to African-American mothers (CDC, 2022).

SCD affects around one in every 16,300 Hispanic Americans, according to some estimates. In the United States, children and adolescents make up around forty percent of SCD patients. The incidence varies greatly depending on the state and the region of the country with the highest ethnic population. Additionally, migration at both the national and international levels impacts the incidence of SCD and HbAS. This is the case in many of the countries that people with SCD and SCA consider to be their homes. A genetic study carried out in Brazil has not only proven a relationship between the lineage of these people and the slave trade in West Africa, but it has also shown that these people may have originated from West Africa (Mina Coast and Angola). Experts predict that the rise in technological advancement and the ease of international travel will increase the prevalence of SCA. By 2050, experts predict that SCA will affect over 400,000 newborns annually (Piel et al., 2013). The rates of death and morbidity in nations with high incomes compared to those with low incomes are significantly different from one another, as well as from one another. The adoption of immunization recommendations for those with SCD and extensive screening procedures has resulted in a substantial drop in the death rate of children with SCD who were under the age of four years old, which has been recorded as a decrease of 68% from 1999 to 2002 when compared to 1983 to 1986. Between 1999 and 2002, we observed this downward trend. On the other hand, between 50 and 90 percent of children under the age of five who are afflicted with SCD in sub-Saharan Africa die before

reaching their fifth birthday. This is a much higher mortality rate than in other regions. The increased emphasis placed on education and training for those working in the medical field has increased the average lifespan. This has led to an improvement in the quality of medical care accessible in countries with high incomes (Piel etal., 2013).

On top of that, this has resulted in an upsurge in the accessibility of medical treatment options. Despite this, it remains years behind cohorts of persons who do not have SCD (54 years as opposed to 76 years for predicted longevity and 33 years as opposed to 67 years for lifestyle-adjusted expectancy). Thirty percent of all SCD patients in the United States suffer from HbSC diseases. People with the HbC feature (heterozygous mutation), in the same way as patients with HbAS do, and remain symptom-free for the bulk of their lives. Although HbSc disease is generally considered a milder type of SCD, it nevertheless has the potential to cause significant morbidities (Howard et al., 2014).

Patho- and Histo-physiology

Sickle cell anemia is marked by RBCs in the peripheral blood that looks like sickles because they are long and narrow at the ends. We also refer to these RBCs as drepanocytes (Serjeant, 1992). Many of the participants' medical records included additional outcomes. Howell-Jolly bodies: People with spleens removed often find these DNA remnants in their red blood cells. Therefore, individuals with SCA undergo auto splenectomy. Target cells, also known as hepatocytes, are the most prevalent in thalassemia patients. Typically, they occur in individuals with sickle-thalassemia syndromes, although they may also occur in individuals with sickle-cell anemia (Steinberg, 1999). In reaction to hemolysis, polychromatic cells, also known as reticulocytes, emerge in the bone marrow as reticulocytes. Occasionally, the peripheral smear may detect nucleated red blood cells. These results do not complement or enhance one another in any manner. The only confirmatory techniques are isoelectric focusing, hemoglobin electrophoresis, and high-performance liquid chromatography (Rees et al., 2010). DNA-based techniques are not used nearly as often as other techniques. Individuals with unclear diagnostic categorization receive these techniques instead. Prenatal

diagnostics utilize amniocentesis-extracted fetal DNA. The extraction of fetal DNA from maternal blood is a topic that is still the subject of current research (Bianchi, 2012).

Epidemiology of Sickle Cell Traits

Individuals with African ancestry or ancestors who lived in areas of the globe classified as tropical or subtropical, where malaria is prevalent, have a greater chance of carrying the sickle cell trait in their genes. In the United States, nine percent of people of African descent are carriers of the sickle cell trait, which means that 9.1% of African Americans have sickle cell disease. It is equivalent to around 3 million people, whereas the prevalence is just 0.2% among Caucasians (Gibson, J. S., & Rees, D. C. 2016). It is estimated that 300 million people globally carry the sickle cell trait, with one-third residing in sub-Saharan African countries (El Ariss et al., 2016). In areas with a higher frequency of malaria, there is also a higher prevalence of the sickle cell trait. Gibson and colleagues estimate that the incidence is as high as sixty percent in Saudi Arabia and as high as twenty-five percent in some regions of Africa. (Gibson & Rees 2016). There will be an increase in the number of people in the Western world who have both sickle cell trait and sickle cell illness due to the rising migration of people from regions with a high incidence of sickle cell diseases, such as Africa and the Middle East (Modell & Darlison, 2008).

Importance of Sickle Cell Traits

One to three million Americans has the inherited blood illness sickle cell trait, and between 8 and 10 percent of those affected are of African American ancestry (Piel et al., 2013). Those of Hispanic descent, South Asian descent, white descent from southern Europe, and Middle Eastern descent may all be impacted by the sickle cell trait to varying degrees. There are about one hundred million individuals around the globe who are afflicted by the sickle cell trait. There is no mild version of sickle cell disease; sickle cell trait, sometimes known as SCT, is the most common name for this condition. The presence of a single gene that may cause sickle cell disease (also known as SCD) and the ability to carry on the sickle cell trait to subsequent generations is referred to as the "sickle cell trait, or SCT for short [NHLBI], 2022).

In most cases, individuals who have SCT do not present with any of the signs and symptoms of SCD, and they can enjoy their everyday lives. The pigment hemoglobin in red blood cells is responsible for giving blood its characteristic red color. It transfers oxygen across the body from one area to another. Alpha- and beta-globin, two proteins that are remarkably similar to one another and "stick together," make up hemoglobin(Rees et al., 2010) For hemoglobin to function in the body, both proteins need to be present and in good working order. Red blood cells from SCT patients include both regular and aberrant hemoglobin. DNA is the code that tells red blood cells how to generate alpha- and beta-globin proteins. These proteins are necessary for oxygen transportation in the body [NHGRI], 2020).

Everyone has two genes that are necessary for the production of beta-globin. They get a single copy of the beta-globin gene from every single one of their biological parents according to the inheritance pattern described above. A person is considered sickle cell trait (SCT) if they receive a sickle beta-globin gene from a single parent but possess a typical beta-globin gene from the opposite parent. Regular beta-globin genes are responsible for producing healthy red blood cells. From this, the individual will not get sickle cell disease but will become a "carrier" of the trait and might pass it on to their children. A hereditary ailment known as sickle cell disease causes the red blood cells to take on a sickle-like shape. Those who have sickle cell trait rather than sickle cell disease, a potentially fatal condition in which patients have two defective genes that result in the synthesis of sickle-shaped hemoglobin (a component of red blood cells that helps red blood cells carry oxygen), have only one defective gene and typically live healthy lives. Sickle cell disease patients have two defective genes that result in the synthesis of sickle-shaped hemoglobin. Those who have the sickle cell trait are at increased risk of experiencing serious health complications, including sudden death, when exposed to extreme conditions such as severe dehydration and strenuous activity. Individuals are said to have a sickle cell trait when they are born with an inherited copy that carries the gene associated with sickle cell disease or if they acquire one from their grandparents. Sickle cell disease is a genetic condition that causes sickle cells to form in red blood cells. It should not be confused with a medical condition. In most circumstances, People with the sickle cell trait may lead healthy lives free of the difficulties that frequently come along

with carrying the trait. The sickle cell trait cannot be the fundamental reason for sickle cell illness in its chronic form. Despite this, parents with sickle cell traits can give their offspring the genes that cause sickle cell disease (Steinberg, 1999).

Clinical Features

Vasoocclusive ischemic attacks and big vessel vasculopathy are the two most common harmful effects of sickle cell disease. Vascular-occlusive ischemic events can cause a lot of pain and damage to organs in the future, like hypersplenism, osteonecrosis, retinopathy, and liver damage. Large-vessel vasculopathy can result in cerebrovascular disease, pulmonary hypertension, and nephropathy, priapism, and leg ulcers. According to Eltzschig and Eckle (2011), vascular occlusion and ischemic damage induce acute, recurring, and excruciating sickle cell crises. This is because of a blockage in the post-capillary venules and damage caused by ischemia and reperfusion. Because of low oxygen levels, ischemia, and the damage they cause to the tissue, mast cells become activated. This causes the production of mediators related to inflammation. However, researchers have identified dehydration, illness, fever, cold, stress, acidosis, hypoxia, and discomfort as potential causes (Ballas, 1998). It is still unknown what causes acute vaso-occlusive pain; nonetheless, they have all been considered as possible explanations. According to Cataldo et al. (2015), intense pain episodes are associated with sickle cell disease because hyperalgesia and activation of peripheral afferent nociceptors contribute to them. Home therapy is generally beneficial for most episodes (Tuijn et al., 2017). As part of the strategy for supportive therapy, the patient will get enough pain medication (such as paracetamol, non-steroidal anti-inflammatory drugs, and opiate analgesia at specific time points), as well as drink, warmth, and rest. Ending the acute painful crises as soon as they begin may be feasible to prevent or at least reduce the amount of tissue damage that occurs (Houwing et al., 2019; Ballas et al., 2012). Patients, as well as their parents or other carers, need to be aware of the warning signals of an acute, painful vaso-occlusive crisis to seek medical help promptly before the crisis leads to more catastrophic repercussions. Patients suffering from acute vaso-occlusive pain who present to the emergency department need prompt triage, evaluation, and stringent administration of analgesics. When traditional techniques

of pain treatment have been ineffective, it may be necessary to use alternative drugs such as ketamine or clonidine (Yawn et al., 2014).

Diagnosis of Sickle Cell Disease

Diagnosing blood illnesses such as sickle cell anemia and other conditions in the early stages of an individual's life is a technique to decrease the detrimental effects of its derangement in people who test positive for the condition. The confirmation process might take place at any point throughout the disease's progression. However, early detection of SCD can effectively reduce the morbidity, treatment, and mortality rates associated with the condition. Patients diagnosed with SCD in affluent nations now enjoy a longer life expectancy because of advances in early diagnosis, intensive treatment, and general medical care. Therefore, early detection of the condition can lead to more effective treatment (Lubeck et al., 2019). The clinical laboratory is mainly responsible for finding sickle cell disease and hemoglobin S. They do this by using molecular testing, biochemical testing, or a mix of the two. According to Zandecki (2007), the most common conventional techniques for detecting sickle cell disease are a complete count of blood (CBC), hemoglobin electrophoresis, and high-performance liquid chromatography (HP liquid chromatography). These are the three most common diagnostic procedures. A sickling test, a total blood count, and peripheral blood films (also known as blood smears) are all examples of screening tests; a solubility test and other advanced diagnostic testing, such as techniques in Hb sunder and analytical genetics, are complex, expensive, and require highly specialized workers to perform them in a centralized laboratory. These tests include total blood counts (Rees et al., 2010).

On the other hand, sophisticated, compact point-of-care devices have been created to provide an option that is simpler, less costly, and more user-friendly for quickly identifying infectious illnesses. According to research by Bawor et al. (2020), some of the most popular diagnostic procedures for sickle cell disease include a complete blood count, hemoglobin ion electrophoresis, and high-performance liquid chromatography. These three approaches are consistently regarded as the most reliable and effective practices in their respective industries. In order to arrive at an accurate diagnosis of sickle cell disease, it is possible to use phenotypic and genetic methods, which include enzyme-based

restriction fragment length polymorphism, also known as RFLP, and polymerase chain reaction (PCR) (Rees et al., 2010).

Current Treatment and Gene Therapy Treatment

Patients diagnosed with sickle cell trait often do not need any treatment. Treatment is not required until and unless the affected individual displays signs of any medical condition, even those conditions connected to the trait. Clinicians are responsible for being aware of the challenges produced by the sickle cell trait to initiate appropriate patient care as soon as symptoms manifest themselves. Individuals who have sickle cell characteristics and come with hematuria, for instance, need to have the possibility of papillary necrosis ruled out as soon as possible. Patients in this situation should get conservative treatment. Sickle cell disease (SCD) is a type of hemoglobin that happens when valine replaces glutamic acid on the ß-globin chain. This illness affects millions of people all over the globe and may cause both short-term and long-term damage to organs. There are presently just a handful of treatment options available for SCD, and even those are limited by factors like cost, accessibility, and individual toxicity (Rees et al., 2010). This is still true even in areas with top medical care and longer lifespan.

Gene Therapy

The most prevalent and severe genetic abnormality is sickle cell anemia, and it affects millions of people all over the world. Only allogeneic stem cell hematopoietic transfusion has demonstrated efficacy in treating the illness. However, scientists are looking for other treatments because related sibling sources are challenging to come by, and there is a high chance that the transplant will not work. Much research has been done on ex vivo gene therapy using the n-n-linked inserting gene, and it is now being looked at in clinical trials. The recent advancements in our knowledge of how molecules function to govern globin switching and erythropoiesis in animals have made novel therapeutic approaches available that hold a tremendous deal of promise. Sickle cell disease (SCD) is a severe form of genetic anemia caused by a single change in the adult hemoglobin (Hb) tetramer. This change makes Hb more likely to polymerize when oxygen levels are low. SCD is another term for sickle cell syndrome (SCD). The sixth codon of the globin chain

is the site of this specific mutation (from glutamic acid to valine). Over one million people in the United States are affected by the long-term and acute signs and symptoms of sickle cell illness, such as periodic pain crises, silent cerebral infarction, stroke, damage to end organs, and early death. Other symptoms of sickle cell illness include anemia and an increased risk of infection. It is believed that several million people throughout the world have sickle cell disease. Sickle cell illness affects a large number of people. Polymerized sickle hemoglobin, sometimes called HbS, is a type of sickle hemoglobin that prevents red blood cells from retaining their flexibility and biconcave design. This interference results in hemolysis, which restricts blood flow, and crescent-shaped cells that adhere more strongly to the vascular endothelium. More in-depth assessments of the pathophysiology and genetics of SCD may be found in the published research, which can be read as extra reading (Ware et al., 2017).

Limitations of the research

The primary limitations of the thesis include the small and specific sample size of 108 Zimbabwean students in Northern Cyprus, which limits generalizability to broader populations. The geographic focus further restricts applicability to other regions. Methodologically, while PCR provides accuracy, it may overlook other genetic factors or mutations influencing hemoglobin variants. The study also does not have enough environmental, historical, or lifestyle data, which could change how alleles are distributed. It also does not have enough health or clinical correlations to look into the effects of the sickle trait. Technical challenges, such as potential contamination or PCR bias, may also impact accuracy. These limitations highlight areas for improvement in future research to enhance the depth and applicability of findings.

Work in this Thesis

This research aims to examine the frequency of sickle cell disease carrier status among Zimbabwean students studying in Northern Cyprus and to highlight the findings. It examines the prevalence of the A (normal hemoglobin) and S (sickle hemoglobin) alleles among 108 Zimbabwean students in Northern Cyprus. The study used DNA extraction, real-time PCR amplification, and genotyping to find that the allele was more

common than the S allele (0.87), but no SS genotypes were found. Statistical analysis confirmed that the results aligned with the Hardy-Weinberg Equilibrium. The study shows sickle cell traits are less common in this group than in other African groups. This information is helpful for genetic counseling and future research.

Significance of the Study

The results of this research will help shed light on the fair distribution of sickle cell characteristics among Zimbabwean students currently enrolled in higher education in Northern Cyprus. In addition, the results of this research will raise awareness among other international students, urging them to follow the safety measures that Zimbabwean students have been taking when they return to their home countries.

Definitions of Key Terms

Sickle Cell Disease - also simply called sickle cell, is a group of hemoglobin-related blood disorders that are typically inherited.

Hemoglobin -Hemoglobin is a protein in red blood cells that carries oxygen. The hemoglobin test measures how much hemoglobin is in your blood.

Hereditary- refers to traits or characteristics that are passed down from parents to their children.

Genotype- The genotype of an organism is its complete set of genetic material

Genetic abnormalities are conditions caused by changes to the genes or chromosomes.

Sickle Cell Trait – is a condition in which a person has one abnormal allele of the hemoglobin beta gene, but does not display the severe symptoms of sickle cell disease that occur in a person who has two copies of that allele.

DNA -deoxyribonucleic acid (abbreviated DNA) is the molecule that carries genetic information for the development and functioning of an organism.

Gene – is the basic physical and functional unit of heredity. Genes are made up of DNA

PCR- is a laboratory technique for rapidly producing (amplifying) millions to billions of copies of a specific segment of DNA

Glutamic Acid -an amino acid used to form proteins

Inclusion and Exclusion Criteria

This thesis, "The Ethical Distribution of Sickle Cell Trait in Zimbabwean Students Studying in Northern Cyprus," looks at how familiar the sickle cell trait is among Zimbabwean students in Northern Cyprus. It focuses on how common the trait is and the ethical issues with genetic testing. It aims to provide insights into the genetic diversity of this student population while ensuring ethical practices such as informed consent, privacy, and participant rights are respected throughout the research process. The inclusion criteria for the study are Zimbabwean students currently enrolled in universities in Northern Cyprus who are willing to provide blood samples for genetic testing and have informed consent. The study period focuses on data from students enrolled during the 2023-2024 academic year. The exclusion criteria include students not of Zimbabwean descent, those not enrolled in universities in Northern Cyprus, or individuals unwilling to participate in genetic testing. Additionally, participants who do not provide informed consent or whose data is incomplete or unreliable are excluded from the study. The thesis highlights the need for ethical, culturally sensitive research practices while contributing valuable knowledge to understanding sickle cell trait distribution in Zimbabwean students in Northern Cyprus.

CHAPTER II

Literature review

Theoretical Framework

Sickle cell disease was first documented in 1910 (Herrick, 1910). Since then, comprehension of the pathophysiology of SCD has advanced, leading to effective treatments that reduce morbidity and death. The characteristic difficulties of sickle cell disease arise from hemoglobin polymerization and the sickled morphology of red blood cells. Sickled red blood cells are stiff, sticky, and longer than normal, which makes blood flow blockages worse and causes them to break down faster. The polymerization of sickle hemoglobin leads to many distinctive phenotypes. Acute consequences include severe pain crises, strokes, acute chest syndrome, splenic sequestration, and an elevated risk of sepsis or other infections, among others (Karnon et al., 2000). The sudden pain crisis, also known as an acute vaso-occlusive event, is the main feature of sickle cell disease, which limits oxygen flow and causes tissue damage. Pain crises are frequent and erratic. Pain crises represent a challenging consequence of sickle cell disease, necessitating that doctors rely on patient self-reports to ascertain the most effective treatment protocols (Claster & Vichinsky, 2003; Wethers, 2000a, 2000b; Wilson, Krishnamurti, & Kamat, 2003). The first sign of vasoocclusion is called "hand-foot syndrome" or dactylitis, which shows up as swelling in the hands and feet of infants with sickle cell disease because blood is pooling and blocking the vessels.

Acute chest syndrome (ACS) is a serious problem in sickle cell disease (SCD) that happens when there are blockages in the lungs, which can include infections. Acute Coronary Syndrome (ACS) may also manifest with symptoms such as fever, chest discomfort, coughing, and cough, and may occur along with other acute symptoms. Getting prompt treatment for acute chest syndrome (ACS) is very important because if it gets worse, it can lead to breathing problems and even death. Splenic sequestration happens when the spleen quickly gets bigger, which leads to less hemoglobin being made and a higher chance of getting infections. In the absence of therapy, some cases may result in mortality.

About 10% of all individuals with sickle cell disease will have a stroke at some point in their life, with the highest frequency happening between the ages of 4 and 6 (Claster & Vichinsky, 2003; Wethers, 2000a, 2000b; Wilson, Krishnamurti, & Kamat, 2003). Strokes

happen when blood vessels in the brain get blocked, which lowers oxygen levels and can cause weakness on one side of the body, headaches, problems with eye movement, and difficulty swallowing. Certain strokes may be subtle and undetected, resulting in diminished learning capacity and functionality without a discernible origin. A common problem for men with SCD is priapism, which is when they have long and painful erections due to blocked blood flow. Nearly 90% of boys with sickle cell disease will have priapism by the age of 20. Untreated prolonged priapism may lead to organ damage and impotence. Renal problems may potentially arise as a result of SCD. The kidneys' fragility renders them susceptible to harm from rigid sickled red blood cells (Claster & Vichinsky, 2003; Wethers, 2000a, 2000b; Wilson, Krishnamurti, & Kamat, 2003). Children with sickle cell disease are also susceptible to gallstones. Surveillance of these organs is essential to avert harm and evaluate surgical or other interventions (Claster & Vichinsky, 2003; Wethers, 2000a, 2000b; Wilson, Krishnamurti, & Kamat, 2003). While there are many major physiological issues for a child with SCD, multiple psychosocial impacts also require attention in therapy. Adolescence presents significant challenges for those with sickle cell disease (SCD). Children with SCD often exhibit delayed growth and development (Wethers, 2000a). The social repercussions of school absences, activity restrictions, and recurrent hospitalizations might induce anguish in a teenager with SCD.

Related literature review

Lervolino et al. (2011) assert sickle cell anemia is the most recognized genetic hematological illness, accompanied by significant consequences. Diagnosis and prompt treatments decrease morbidity and death. The advantages have led to the extensive implementation of newborn screening education campaigns. The National Neonatal Screening Program in Brazil, created by order 822/01, included sickle cell disease within the list of conditions assessed using the "heel prick test." Consequently, researchers have frequently released nationwide studies evaluating the outcomes of this initiative. We conducted a literature review to assess the prevalence of sickle cell trait and sickle cell anemia using data from national newborn screening studies of hemoglobin S (Hb S). A literature review was conducted using the keywords sickle cell anemia, hemoglobinopathies, newborn screening, and Brazil in the Bireme and SciELO databases. We examined Brazilian studies that provide data on the prevalence of sickle cell trait (Hb AS) and sickle cell anemia (Hb SS) derived from newborn screening for Hb S. Twelve original national studies were discovered, revealing prevalences ranging from 1.1% to 9.8% for the sickle cell trait and from 0.8 to 60 per 100,000 live births for sickle cell

illness throughout several Brazilian areas. Conclusion: Neonatal screening for Hb S is an effective tool for evaluating the prevalence of sickle cell trait (Hb AS) and sickle cell anemia (Hb SS) in Brazil. The illness has a varied distribution, with the greatest frequency in the northeastern area and the lowest in the southern region.

Okwi, E. L., et al. (2010) The first study on sickle cell disease (SCD) conducted in Uganda in 1949 indicated that the Bundibugyo area in Western Uganda had the greatest prevalence of sickle cell trait (SCT) at 45%. This tower is considered to be the tallest on the whole planet. The study indicated that the prevalence of SCT in the Mbale and Sironko districts in the East was 20-28%, but in the Mbarara and Ntungamo districts in the West, it was 1-5%. There have been no follow-up surveys conducted over the past 60 years. Sudden cardiac arrest (SCA) constitutes almost 16.2% of all pediatric fatalities in Uganda. The inheritance pattern of SCT, however, forecasts probable changes in its incidence and distribution. The aim of the research was to determine the current prevalence of SCT in Uganda. The research used a cross-sectional survey conducted in the districts of Mbale and Sironko in Eastern Uganda, as well as Mbarara/Ntungamo and Bundibugyo in Western Uganda. The participants consisted of youngsters aged 6 months to 5 years. Blood samples were obtained from each participant and assessed for hemoglobin S using cellulose acetate electrophoresis. The determined prevalence of SCT (As) in Eastern Uganda was 17.5%, in contrast to 13.4% in Bundibugyo and 3% in Mbarara/Ntungamo. 1.7% of children in Eastern Uganda tested positive for hemoglobin SS, compared to 3% in Bundibugyo, resulting in gene frequencies of 0.105 and 0.097 for the recessive gene, respectively. No SS was identified in Mbarara/Ntungamo. Many biological and social variables may be responsible for the dramatic change in the incidence of SCT and SS in Uganda. This research provides data suggesting that intermarriages may reduce the incidence of SCT.

Adam et al. (2019) estimated that 50% to 90% of babies born with sickle cell anemia (SCA) in sub-Saharan Africa perish before the age of five. Northern Darfur State in western Sudan has a multiethnic population with a significant prevalence of sickle cell anemia; however, less information is available on the subject. This research sought to ascertain the frequency of sickle cell anemia among Minors admitted to Al Fashir Teaching Hospital in Al Fashir, Northern Darfur State, Sudan. Among these 400 pediatric patients, hemoglobin electrophoresis determined the incidence of sickle cell disease to be 59 (14.8%). Patients with sickle cell trait constituted 11.3%, whereas those with sickle cell disease represented 3.5%. Individuals with SCA have persistently low hemoglobin concentrations, normal mean corpuscular volume, and elevated mean white blood cell

counts. Individuals with sickle cell trait had hematological characteristics comparable to those of normal individuals.

Brandelise et al. (2004) investigated the Newborn screening for sickle cell disease, which began in 1992 in São Paulo State. By the end of 2000, the program included 78 institutions across 36 municipalities, screening a total of 281,884 infants. Initially reliant on liquid cord blood samples, they are being supplanted by dried filter paper capillary samples to facilitate handling and mitigate diagnostic ambiguity due to maternal contamination. The prevalence of sickle cell trait (2.0%) and HbC trait (0.6%) dramatically rose from 1996 to 2000, seemingly due to enhanced identification rather than the subsequent establishment of institutions catering to individuals with elevated trait frequencies. There were 29 infants with homozygous sickle cell SS illness and 26 with sickle cell-haemoglobin C (SC) disease, the latter greatly beyond expectations and maybe due to a nonrandom selection of spouses. Sickle cell-b thalassaemia syndromes were more prevalent than in Jamaica, maybe because to interactions with other Brazilian groups possessing higher frequencies of the b thalassaemia gene. The prevalence of defective hemoglobins in this group is lower than in Jamaica; yet, clinically serious sickle cell disease manifests in one out of every 5527 births, which is equivalent to the rates of other notable inborn metabolic disorders. Pinto et al. (2019) state that since 2001, the Brazilian Ministry of Health has been overseeing a National Neonatal Screening Program (NNSP) that encompasses all 26 states and the Federal District of Brazil, aiming to identify six diseases, including sickle cell disease (SCD) and other hemoglobinopathies.

In 2005, the program's coverage reached 80% of the total live births. Since then, it has fluctuated between 80% and 84% internationally, with variations within states (exceeding 95% in São Paulo State). The Ministry of Health has issued many guidelines for the clinical follow-up and treatment of disorders included in the newborn screening program. The primary difficulty remains the organization of the public health network (SUS), including diagnosis and basic treatment through to reference centers, to provide complete care for individuals identified via newborn screening, particularly those with SCD. We have made significant progress, establishing a network within SUS and incorporating scientific and technological advancements into therapy methods. The objectives for the management of SCD patients include enhancing the information disseminated to healthcare professionals and patients, implementing strategies to prevent complications, and promoting health and care while considering these patients holistically to decrease mortality and improve their quality of life.

Sabarens et al. (2014) assert that children in impoverished nations afflicted with sickle cell disease (SCD) have elevated mortality rates, particularly in some regions of Africa. They compare the five-year projected death rate among children born from 1999 to 2001 with that of children born from 2009 to 2011. From 1998 to 2012, sickle cell disease was identified in 2,591 out of 3,617,919 neonates examined in Minas Gerais, Brazil (1 in 1,400). The estimated mortality probability [1 - Survival] was determined using the Kaplan–Meier technique. The log-rank test was used to compare survival statistics among groups. Out of 2576 children (15 eliminated), 193 died (7.4%): 153 (79.3%) had SS/Sb0-thalassemia, 34 had SC (17.6%), and six (3.1%) had Sbz thalassemia. The 5-year estimated mortality rate for infants born between 2009 and 2011 (n=509) was lower than that for those born between 1999 and 2001 (n=624), but the difference was not statistically significant [mean (SD) 5.8% (1.1) vs 6.2% (1.0)]. Notwithstanding a robust and extensive screening program, mortality from SCD in Minas Gerais remains high. To reduce death rates, socio-economic development and education programs on SCD for healthcare professionals and families are essential.

Naik and Haywood Jr. (2015) argue that the sickle hemoglobin (HbS) point mutation has experienced independent evolutionary selection at least five times globally due to its significant protective benefits against malaria in the heterozygous condition. In 1949, homozygous Hb S, or sickle cell disease (SCD), was the first hereditary disorder discovered at the molecular level; nevertheless, both SCD and heterozygous Hb S, known as sickle cell trait (SCT), have subsequently had a protracted and intricate history. The rapid deployment of early mass screening programs for sickle cell disease (SCD), the recent introduction of focused screening regulations for sickle cell trait (SCT) in sports, and apprehensions about stigmatization have generated significant debate around research and policy issues related to SCT. While SCT mostly provides protection against malaria, afflicted carriers may have clinical consequences, including exercise-induced injuries, renal problems, and venous thromboembolism. The historical context of SCD and SCT has imparted insights on conducting contemporary research to reduce stigmatization, enhance study outcomes, and guide genetic counseling and policy formulation for SCT.

Shanna Lea (2003) states that carriers of sickle cell trait are healthy; yet, they face the chance of having offspring with sickle cell disease (SCD), a severe hematologic condition. The ineffective population screening for sickle cell trait (SCT) has led to a significant number of African American adults reaching reproductive age unaware of their risk. Recent experiences with newborn screening follow-ups for hemoglobinopathies indicate

that interest in genetic screening for sickle cell traits is minimal. This research seeks to enhance the acceptability of genetic screening within the African American community by implementing an educational program and evaluating the existing cultural health attitudes around sickle cell disease. This work is crucial for public health since sickle cell disease is the predominant genetic illness impacting the African American population, and educational initiatives to encourage screening must be attuned to the community's cultural beliefs. The impact of education on sickle cell disease and the acceptability of genetic screening for the trait has been evaluated by anonymous surveys administered to female African American patients in a high-traffic prenatal clinic. We used the Health Belief Model to evaluate the prevailing health beliefs around SCD and trait testing through anonymous questionnaires.

The research has shown that a short educational intervention on SCD in a prenatal context effectively enhances understanding of SCD and acceptability of SCT screening (p-value < 0.001). African American women who can have children tend to believe that sickle cell disease (SCD) is very serious, think they are less likely to be at risk for SCD, strongly support sickle cell trait (SCT) testing, and see few barriers to getting tested for SCT. Education in a prenatal context may serve as a framework to enhance the acceptability of screening for SCT. A comprehensive understanding of SCD correlates with increased acceptability; yet, the Health Belief Model indicated that most participants now do not see themselves as personally at risk of having a kid with SCD, irrespective of their awareness of the condition. The future teaching of SCD must consider these misconceptions in order to properly stimulate interest in SCT testing.

CHAPTER III

Methodology

Materials Used

DNA isolation Kit (Hibrigen Biotechnology LTD, Gebze, Turkey), Oligos (Macrogen, Seoul, South Korea), 2xTaqMan Master Mix (Hibrigen Biotechnology LTD, Gebze, Turkey), HiMedia Q96 Plus (Himedia, PA, USA), Nanodrop ND200 Spectrophotometer (Thermo Scientific and located in Waltham, Massachusetts, United States).

Sample Collection

At the Near East University Hospital Laboratory, blood samples from 108 randomly chosen Zimbabwean students was taken with a glass syringe and put in 2.5-ml Ethylenediaminetetraacetic acid (EDTA) tubes.

Computers

The PC computer system in its entirety, including all software packages XP version of Microsoft Office.

Human DNA

Blood samples were used to extract genomic DNA from 108 randomly selected Zimbabwean students, which was then processed in a class II laminar flow hood using the appropriate pipettes. During the process of extraction, this step was taken so that there would be as little chance of contamination as possible. In order to extract and purify genomic DNA from whole blood, the procedure outlined by the business that manufactures DNA Isolation Kit (Hibrigen Biotechnology LTD, Gebze, Turkey) was used.

Measurement of DNA Concentration

At 260 and 280 nm, a DNA concentration was determined using a Nanodrop ND200 Spectrophotometer (Thermo Scientific and located in Waltham, Massachusetts, United States).

Oligonucleotides

Table 1.

A table showing sequence of gene and base pair (Oligonucleotides)

HbS_M	GCAGTAACGGCAGACTTCTCCT
HbS_WT	GCAGTAACGGCAGACTTCTCCA
HbS_P	FAM-GGAGCAGGGAGGGCAGGAGCCAGG-BHQ1
HBB_MC_F	TGCCAGAAGAGCCAAGGACA

The first shows the revers primer for mutant allele, the second is the revers primer for wild type allele, the third shows the probe, and the last is the forward primer.

Methods

DNA PCR Amplification and Genotyping

Allele-specific primers for HbS mutation (*HBB* 20A>T (p. Glu6Val) was designed using SNAPgene software. Real-Time Polymerase Chain Reaction (RT-PCR) was conducted using 2xTaqMan Master Mix (Hibrigen Biotechnology LTD, Gebze, Turkey).

PCR mixture

The PCR mixture was made by centrifuging 12.5 µl of commercially acquired Thermos Scientific PCR master mix 2X MM. The PCR master mix was purchased from the market. In order to get an overall measurement for the PCR cocktail, 2.50 µl of DNA was required, 1µl deionized water, 0.5 of µl forward primer, 0.5 µl of reverse primer, and 0.5 ul of the probe. Additionally, 1µl dH20, 0.5 µl M of the forward primer was used. This

was carried out for 35 cycles, employing the three primary procedures involved in PCR amplification, as shown in *figure 2.*

Figure 2.

A PCR mixture that was used for the study

Components	1 X
Taqman Master mixed	12.5µl
Forward Primer	0.5µM
Reversed Primer	0.5µM
Probe	0.25µM
dH_20	1.0 µl
DNA	2.5 µl

DNA PCR Amplification

The PCR process involves three main amplification stages, repeated over 35 cycles. During the denaturation stage, the thermal cycler heats the solution in the tube to at least 95°C for one minute. This heat breaks the hydrogen bonds in the original DNA sample, separating it into single strands, a process known as the denaturation of double-stranded DNA. Next, the annealing stage begins. For thirty seconds, the temperature is lowered to between 50°C and 60°C (122°F and 140°F). This lets the DNA primers and DNA polymerase enzyme attach to the heat-separated DNA strands. This step is known as primer annealing. During the extension stage, which lasts one minute at 72°C and involves nucleotides (A, T, C, and G) from the solution are paired with each DNA strand. This process generates new complementary DNA strands, resulting in duplicate double-stranded DNA molecules from the original single strands. The temperature fluctuates between 95°C for denaturation and 50°C–60°C for annealing. The thermal cycler repeats these heating and cooling cycles 35–40 times, doubling the DNA sequence with each cycle. A single short DNA sample produces millions of copies of the original DNA fragment by the end of 35 cycles**.**

Figure 3.

A figure showing a DNA PCR Amplification cycles

State	Temperature	Time
Denaturation	95 °C	1minute
Annealing	57 °C	30 sec
Extension	72	1 minute

To minimize the risk of contamination, the DNA amplification process was conducted in a laminar flow hood that had been sterilized with 95% alcohol (v/v). Additionally, all equipment was thoroughly cleaned to reduce contamination risks further. The DNA was amplified to generate an exponentially increasing number of copies. All tubes were adequately sealed, labeled, and centrifuged briefly before being placed in a thermocycler programmed for PCR amplification. The thermocycler was set to operate for 35 cycles, utilizing the three primary stages of PCR amplification. The preliminary denaturation was performed at 96°C for three minutes. Subsequently, the mixture underwent 35 cycles, each consisting of heating at 96°C for 20 seconds, annealing at 60°C for 30 seconds, and extension at 65°C for one minute and 30 seconds. Several fluorescent dyes were employed as FAM channels to identify sample mutations.

Statistical analysis

The Hardy-Weinberg equilibrium (HWE) was assessed using the Pearson Chi-Square (χ^2) test to determine genotype distributions and allele frequencies. A p-value of less than 0.05 was regarded as statistically significant. The exact HWE test was conducted using the website https://www.cog-genomics.org/software/stats, and the results were interpreted using the GraphPad Prism program (GraphPad Software, Inc., San Diego, California, United States).

Duration of the Study

January 2022-December 2024

CHAPTER IV

Findings and Discussion

Introduction

A missense point mutation (HBB: 20A>T, p.Glu6Val) results in the substitution of glutamic acid with valine at the sixth amino acid position (Creary et al., 2007). When present in a homozygous state, this single nucleotide pathogenic variation leads to a severe chronic condition characterized by multi-organ complications. However, individuals who are heterozygous for this mutation are generally asymptomatic and exhibit resistance to malaria infection (Nietert et al., 2002).

Approximately 5.2% of the global population carries pathogenic variants of the *HBB*gene (Modell et al., 2008). Although the sickle cell mutation is most prevalent in Africa, individuals from Italy, Turkey, Greece, the Middle East, and India may also carry the p.Glu6Val mutation or other variations in the globin gene (Serjeant, 1992; Hickman et al., 1999). According to International Hemoglobinopathy Research Network (INHERENT), 54.8% of the 73,200 individuals affected by hemoglobinopathies fall into one of three categories: HbS/HbS, HbS/β, or HbS/HbC. This accounts for approximately 53,100 individuals with Hb S-associated disorders (INHERENT, 2021).

Sickle cell anemia is highly prevalent among newborns in western and central Africa, affecting about 1% to 2% of all births. Additionally, the sickle cell carrier rate averages around 28% across western, central, and eastern Africa (Frempong et al., 1994; Kountouris et al., 2021). In the Middle East including countries such as Saudi Arabia, Sudan, Tunisia, Kuwait, Lebanon, and Yemen the estimated carrier frequency for Hb S is 2.5%, with approximately 162 children expected to be born with sickle cell syndromes annually (Weatherall & Clegg, 2001).

Individuals of African descent or those with ancestors from tropical and subtropical regions where malaria is endemic have a higher likelihood of inheriting the sickle cell trait. In the United States, approximately 9% of African Americans carry the sickle cell trait, leading to a sickle cell disease prevalence of about the same percentage within this population (Hassell, 2010). This equates to roughly 3 million affected individuals,

whereas the prevalence among Caucasians is significantly lower, at only 0.2% (CDC, 2020).

Jonathan and Rees 2016 An estimated 300 million people worldwide carry the sickle cell trait, with one-third living in sub-Saharan African countries Matar & Berjaoui (2016). In areas with a higher frequency of malaria, there is also a higher prevalence of the sickle cell trait. Gibson and colleagues estimate that the incidence is as high as sixty percent in Saudi Arabia and as high as twenty-five percent in some regions of Africa. (Jonathan & Rees 2016). So many people are moving to developed countries from places like Africa and the Middle East, where sickle cell diseases are common. As a result, more people in these countries will have sickle cell traits and sickle cell disease. This will lead to a rise in the proportion of people with either sickle cell traits or illnesses (Piel et al., 2017). This research investigates the prevalence of the sickle cell trait among the Zimbabwean population. A hereditary predisposition causes a high incidence of sickle cell disease (SCD) in that region (Grosse et al., 2011).

Dr. J.B. Herrick discovered the finding in 1904 when he was examining the blood of an anemic West Indian medical student (Herrick, 1910). It is uncertain exactly when, how, or where the mutation that creates the sickle cell gene took place. However, scientists hypothesize that the Veddoids on the Arabian Peninsula in the Middle East are the origin of this mutation (Piel et al., 2017). Over time, the gene responsible for sickle cell anemia spread to various regions of the globe, including India, southern Europe, and Africa. Those of African heritage and those living in tropical Africa, southern Europe, and the Middle East are the most likely to have the sickle cell trait (Grosse et al., 2011).

Demography of the studies group

For this study, 108 sample Zimbabwean students enrolled at the Near East University in Northern Cyprus were selected.

FIGURE 4.

Demographic Findings of the Studied Population with respect to their genders

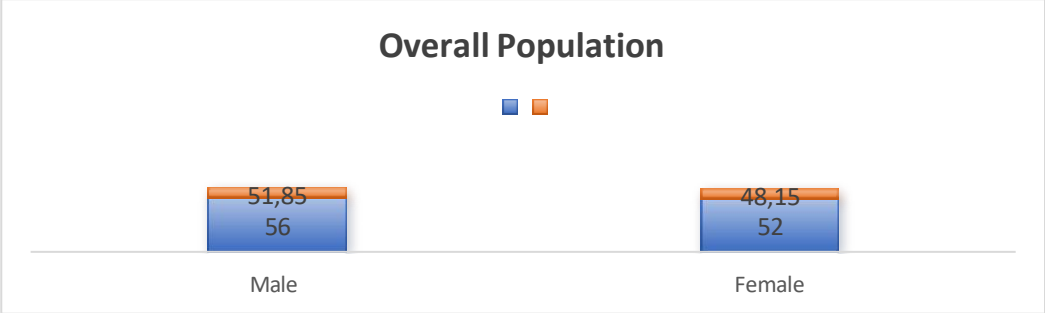


FIGURE 5.

Distribution of age range in the *studied* population

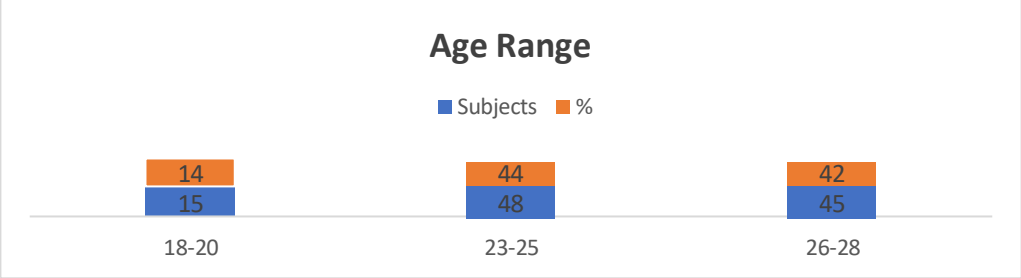


Figure 4 displays the overall population of the study group and it also points out that the males that were studied were more than the females; the total number of males was 56, resulting in a percentage of 51.85, while the total number of females was 52, displaying a percentage of 48.15. This resulted in a total of 108 samples.

Figure 5 shows the age range of our sample size, which ranged from eighteen to twenty-eight years (18–28).

The *HBB* gene amplification

Beta-globin is a protein produced by the *HBB* gene, which provides instructions for its production (Steinberg, 1999). Beta-globin is a subunit of hemoglobin, a more significant protein in red blood cells. Hemoglobin is responsible for carrying oxygen throughout the body. Adult hemoglobin has four distinct protein components: two beta-globin subunits and two alpha-globin subunits. The HBA gene is responsible for the production of alpha-globin (Weatherall & Clegg, 2001). A heme molecule containing iron attaches itself to each protein subunit. The iron at the center of each heme can covalently bond with a single oxygen molecule. Hemoglobin in red blood cells binds to oxygen

molecules in the lungs. These cells then enter the circulatory system, transporting oxygen to tissues throughout the body (Ingram, 1957). A genetic mutation in both copies of an individual's *HBB* gene causes Sickle Cell Disease (SCD), an inherited condition (Serjeant, 2013). This mutation affects hemoglobin, crucial for oxygen delivery across the body. The mutation causes hemoglobin molecules to clump together, giving red blood cells a sickle shape. These sickle-shaped cells can lead to ruptured blood cells, anemia, chronic pain, weakened immune function, organ damage, and even early death. Bone marrow transplantation can potentially treat SCD, but finding suitable donors is often challenging, and the procedure carries significant risks of hazardous side effects (Weatherall & Clegg, 2001). A single-point mutation that substitutes valine for glutamic acid causes Sickle cell disease, the most common naturally occurring *HBB* mutation (Ingram, 1957). Sickle cell anemia is the most common form of SCD and is one of hundreds of inherited *HBB* variants identified.

This mutation leads to hydrophobicity on the cell's exterior, causing hemoglobin molecules to polymerize. This morphological change impairs the red blood cells' functionality, culminating in the complete manifestation of SCD, regarded as one of the most fatal genetic disorders (Serjeant, 2013). According to research by Pawloski et al. (2001), individuals heterozygous for SCD, also known as carriers, do not exhibit symptoms of most infections caused by Plasmodium, including malaria (Steinberg, 1999).

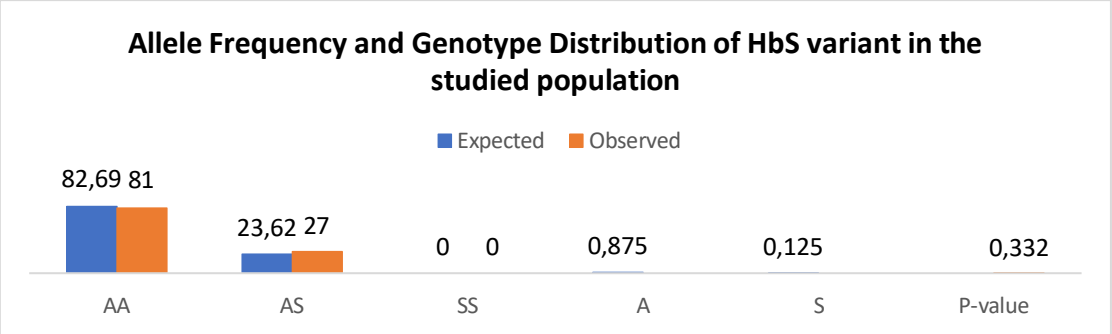
Genotyping

Genotyping refers to determining a genotype or DNA sequence at specific sites on the genome (Browning & Browning, 2011). We can use sequence variations as markers to identify genes linked to specific characteristics or conditions. Genotyping is a method that determines the degree to which individuals and communities differ in the DNA sequences they possess. An individual's genotype may be defined by comparing their genome to reference genomic sequences for the general population as a standard (Alberts et al., 2014). There are various ways to differentiate a variant sequence from a reference sequence. Examples of genetic variations include insertions and deletions, also known as indels; single nucleotide polymorphisms, or SNPs; single nucleotide variants, or SNVs; and copy number variation (Benjamin, 2020). Understanding the relationship between

phenotypic and genetic characteristics is possible through genotyping. Hidetoshi (1997) performed genotyping within a family using PCR analysis to discover Hb variations. The genotyping process utilized five different restriction enzymes. Is one of the restriction enzymes used, and are the results producing informative bands? A study group concluded that molecular restriction endonucleases (PCR) may help with genotyping genetic variations because of these results (Hidetoshi, 1997)."Homozygous" refers to a genotype where the individual's alleles are identical. "Heterozygous" refers to a genotype in which both alleles present are different. The genotype affects the phenotype, which consists of observable characteristics and traits of the person or organism (Benjamin, 2020). The extent to which a person's genotype influences their phenotype depends on the characteristic. Genotype is the only factor that may affect the color of the petals on a pea plant, for instance. The color of the petals can range from white to purple depending on the alleles present in the pea plant (Alberts et al., 2014). Conversely, a person's genetic makeup only moderately impacts specific characteristics. These characteristics are typically called complex traits because various other factors influence them, including environmental and epigenetic influences (Browning & Browning, 2011). Due to the impact of environment and growth conditions on appearance and behavior, people who share the same genotype do not always appear or behave the same way. Similarly, there is no guarantee that two creatures with identical outward appearances have the same genotype (Alberts et al., 2014).

Figure 6.

Distribution of Allele frequency and genotype distribution

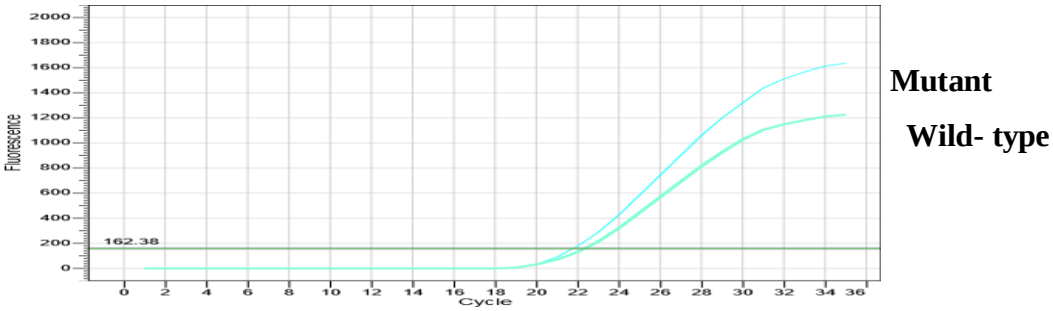


As seen in this Figure, the expected value of individuals with normal hemoglobin (AA) was 82.69. The observed value 81 (75%) had normal hemoglobin cell,the chart shows that the observed value was lower than the expected. The value for AS trait was higher than the expected, while 27 (25%) was observed rather than 23.62 which means

more individuals possess the traits than expected. Allele frequency analysis shows that the normal hemoglobin (A) allele is dominant at 87.5% (0.875), while the sickle cell (S) allele is less common at 12.5% (0.125). The p-value of 0.332 suggests no significant difference between expected and observed genotype frequencies, indicating that the population follows Hardy-Weinberg equilibrium. This implies that evolutionary forces such as selection, mutation, or genetic drift are not significantly influencing the HbS variant distribution.

Figure 7.

PCR Amplification Curve for Mutant and Wild- type



The graph serves as a powerful representation of a qPCR (quantitative Polymerase Chain Reaction) experiment, a cutting-edge technique crucial for quantifying DNA by monitoring fluorescence throughout amplification cycles (Heid et al., 1996). This technique plays a significant role in research by accurately determining DNA quantities. The x-axis represents the number of PCR cycles, while the y-axis displays fluorescence intensity, which increases as DNA is amplified. A clearly defined horizontal threshold line at 162.38 fluorescence units indicates the point where fluorescence significantly exceeds background noise, enabling the precise determination of the Cycle Threshold (Ct) value (Kubista et al., 2006).

The graph features two distinctive curves one in light blue and the other in green each representing different samples or reactions. The light blue curve crosses the threshold sooner, signifying a higher initial concentration of DNA, whereas the green curve crosses later, indicating a lower initial concentration (Kubista et al., 2006). This variation in Ct values is instrumental for comparing initial DNA quantities across samples, providing valuable insights into the genetic material present in each reaction.

The qPCR process occurs in three distinct phases. The baseline phase (cycles 0–18) shows low and stable fluorescence levels, representing background noise as significant DNA amplification has not yet begun (Arya et al., 2005). Following this, the exponential phase (cycles ~20–30) exhibits a sharp increase in fluorescence, marking the exponential amplification of DNA. This phase is indispensable for accurately quantifying initial template amounts since the doubling of DNA during each cycle is most pronounced here. Finally, the plateau phase (cycles>30) occurs when amplification diminishes and stabilizes due to the depletion of essential reagents like primers and dNTPs. Fluorescence reaches a plateau, indicating the conclusion of the reaction (Arya et al., 2005).

The light blue curve passionately represents a sample with a higher starting quantity of target DNA, crossing the threshold in fewer cycles and yielding a lower Ct value (Kubista et al., 2006). Conversely, the green curve illustrates a sample with a lower initial DNA concentration, crossing the threshold later and resulting in a higher Ct value. Ultimately, this graph effectively visualizes the dynamics of DNA amplification across various samples and underscores the significant relationship between Ct values and initial DNA quantities. It highlights the importance of qPCR in molecular biology for understanding genetic variations, gene expression, and the quantification of nucleic acids in different research and diagnostic contexts (Heid et al., 1996).

The Determination of A and S alleles

To find the A allele for normal hemoglobin and the S allele for sickle hemoglobin, DNA from blood samples must be amplified, and analyzed to find specific genetic differences in the *HBB* gene (Rees et al., 2010). Blood is taken in EDTA tubes to inhibit coagulation, and DNA is extracted using specialist tools (Steinberg, 2008). We then assess the purity and concentration of the DNA to ensure its quality for further analysis. Using allele-specific primers that target the normal A allele (GAG, which codes for glutamic acid) and the mutant S allele (GTG, which codes for valine), the polymerase chain reaction (PCR) makes copies of the part of the *HBB* gene that has the mutation (Ingram, 1957). The DNA that has been amplified is checked using techniques like agarose gel electrophoresis, which shows specific banding patterns that show whether the DNA is from an A or S allele, or real-time PCR with fluorescent probes for accurate quantification

(Chan et al., 2011). Sequencing can clearly show the exact nucleotide sequence, telling the difference between genotypes AA (homozygous regular), AS (carrier), and SS (homozygous sickle cell). Alternatively, restriction enzyme analysis is used, whereby enzymes cleave DNA variably based on the existence of the mutation (Weatherall & Clegg, 2001). The gene-counting approach determines allele frequencies by dividing the count of each allele by the total number of alleles detected in the population. The Hardy-Weinberg Equilibrium model guarantees the accuracy of genotype distributions (Hartl & Clark, 2007). The Chi-Square test, a statistical validation method, assesses the conformity of observed data with anticipated genetic patterns (Weir, 1996). This thorough method accurately identifies A and S alleles, hence supporting clinical diagnosis, population research, and genetic counseling (Piel et al., 2013).

Research Result

The PCR genotyping findings revealed that there was no one with the SS genotype (sickle cell disease), according to the genotypic distribution, which shows that 81 samples of the population that was studied had the AA genotype (normal hemoglobin) and 27 samples had the AS genotype (sickle cell trait) (Piel et al., 2013). The A allele frequency was assessed to be 0.87%, whereas the S allele frequency was estimated to be 0.12% among the Zimbabwean students investigated in the Northern Cyprus population (Rees et al., 2010). The gene-counting method was used to find out genotype distributions and allele frequencies (Frankel & Sudoyo, 2014). The goodness-of-fit 2 test was used to see how well they fit the Hardy-Weinberg equilibrium, with a P value of less than 0.05 indicating much disequilibrium. The genotype distributions of the HBB 20A>T (p. Glu6Val) gene were consistent with the Hardy-Weinberg equilibrium ($P = 0.332$). Meaning no effect was observed.

CHAPTER V

Discussion

Introduction

Sickle cell disease (SCD) is a genetic disorder caused by mutations in the *HBB* gene, which encodes hemoglobin, the oxygen-carrying protein in red blood cells. These mutations are inherited from parents and can lead to severe health complications. It is estimated that between 300,000 and 400,000 babies are born with SCD each year, with the majority of cases occurring in sub-Saharan Africa (Kato et al., 2018).

SCD arises when hemoglobin molecules containing the mutant sickle globin units polymerize, causing red blood cells to become rigid and assume a characteristic sickle shape. These sickled cells are prone to breakdown (hemolysis) and can obstruct blood flow, leading to various complications (Kato et al., 2018). In addition to hemolysis, other pathophysiological processes, such as immune system activation and vasoconstriction, contribute to disease severity (Rees et al., 2010).

The phenotypic variability of SCD distinguishes it from other genetic disorders, as its clinical presentation can range from mild to severe. Acute complications include stroke, acute chest syndrome, and severe pain episodes, while chronic conditions such as kidney disease can cause long-term organ damage (Ware et al., 2017). Treatment options such as hydroxyurea (hydroxycarbamide), blood transfusions, and hematopoietic stem cell transplantation can help manage the disease. Early diagnosis significantly improves patient outcomes, and some countries have implemented universal newborn screening programs. However, implementing such programs remains a challenge in low-income regions with a high disease burden (Piel etal., 2017).

SCD disproportionately affects African Americans in the United States and is more prevalent in specific geographic regions worldwide (Brousseau et al., 2010).

This study examined the prevalence of sickle cell trait among 108 Zimbabwean students studying in Northern Cyprus using polymerase chain reaction (PCR) testing. PCR is a highly sensitive molecular technique that amplifies DNA to detect genetic variations accurately (Valentina et al., 2021).The amplified products were analyzed using real-time

PCR to determine whether individuals carried the normal (AA), heterozygous (AS), or homozygous (SS) genotype. The PCR genotyping findings revealed that there was no one with the SS genotype (sickle cell disease), according to the genotypic distribution, which shows that 81 samples of the population that was studied had the AA genotype (normal hemoglobin) and 27 samples had the AS genotype (sickle cell trait) (Piel et al., 2013). The A allele frequency was assessed to be 0.87%, whereas the S allele frequency was estimated to be 0.12% among the Zimbabwean students investigated in the Northern Cyprus population (Rees et al., 2010). The gene-counting method was used to find out genotype distributions and allele frequencies (Frankel & Sudoyo, 2014).This research contributes to a growing body of knowledge on genetic variability in SCD by identifying key genes that influence clinical presentation (Valentina et al., 2021). The use of molecular techniques, such as PCR genotyping, provides a reliable approach for confirming the presence of sickle cell-related mutations and understanding their distribution in different populations.

Prevalence and Challenges of Sickle Cell Disease in Liberia

Sickle Cell Disease (SCD) represents a critical public health challenge in Liberia and other sub-Saharan African countries. Its burden is influenced by genetic factors, resource limitations, and systemic gaps in healthcare delivery. This section examines the prevalence of SCD in Liberia, the health challenges associated with the condition, ongoing efforts to address these issues, and potential pathways for improvement. Liberia, situated in a malaria-endemic region, has a high prevalence of the sickle cell trait (HbAS), estimated to affect 20–30% of the population (World Health Organization [WHO], 2022). This prevalence aligns with rates observed in neighboring West African countries, where the HbAS trait has been evolutionarily maintained due to its protective effect against severe malaria. SCD itself, which occurs when an individual inherits two copies of the sickle cell gene (HbSS), is less prevalent but remains a significant health concern. It is estimated to affect 1–2% of the Liberian population, though actual figures may be higher due to underreporting and the absence of systematic screening programs (Doe et al., 2021). Additionally, high rates of early mortality in undiagnosed children further complicate efforts to accurately quantify the burden of SCD.

The health burden of Sickle Cell Disease (SCD) in Liberia is profound, particularly among children under five years of age. Common complications include:

Severe Anemia: Frequent hemolysis (destruction of red blood cells) leads to chronic and acute anemia, which can be life-threatening without timely interventions such as blood transfusions (Grosse et al., 2011).

Infections: Children with SCD are particularly vulnerable to infections due to functional asplenia (loss of spleen function) caused by repeated splenic damage from sickled cells (Smith & Johnson, 2020). Malaria, pneumonia, and sepsis are leading causes of mortality in children with SCD (McGann et al., 2013).

Pain Crises: Vaso-occlusive episodes, caused by the obstruction of blood vessels by sickled cells, result in severe pain and long-term complications such as bone and joint damage (Piel et al., 2017).

Organ Damage: Chronic episodes of sickling and impaired blood flow lead to progressive damage to vital organs such as the kidneys, liver, lungs, and brain (Weatherall & Clegg, 2001).

Diagnosis of SCD in Liberia is often delayed due to limited access to healthcare facilities and a lack of awareness about the disease. Most cases are diagnosed only after severe complications arise, such as life-threatening anemia or recurrent infections (Grosse et al., 2011).

Socioeconomic and Systemic Challenges

Beyond health implications, SCD imposes significant social and economic challenges. Families often bear the financial burden of frequent hospital visits, blood transfusions, and treatments such as hydroxyurea, which remain scarce and expensive in Liberia (World Health Organization, 2022). Furthermore, stigma surrounding genetic diseases can lead to social exclusion for affected individuals and their families. The healthcare system faces critical infrastructure, human resources, and funding gaps. Newborn screening, a cornerstone of early detection, is largely unavailable. Training for healthcare providers in SCD management is limited, and the availability of diagnostic tools such as electrophoresis is insufficient, particularly in rural areas (Doe et al., 2021).

Prevalence and Challenges of Sickle Cell Disease in Ghana

Like other countries in sub-Saharan Africa, sickle cell disease is a significant public health problem in Ghana. Ghana has one of the highest SCD birth prevalences in the world; the annual incidence is approximately 15,000-20,000 new cases, which represent 2% of live births (Aygun & Odame, 2012). The prevalence of this trait in Ghana is due to the considerable proportion of sickle cell trait present in the population; as high as 15-25% of Ghanaians (Ghana Ministry of Health, 2021).The trait remained in the population due to the evolutionary benefit such populations had in malaria-stricken areas where the trait ensured moderate shielding from severe malaria disease (Williams et al., 2005). In Ghana, the impact of SCD goes beyond its prevalence. This condition is among the highest contributors to both the morbidity and mortality of children. Those affected often suffer from severe hematologic anemia due to massive red blood cell destruction, multiple infections, loss of immunity, and painful episodes of blocked blood flow, known as vaso-occlusive crises. Due to these complications, many patients will frequently be readmitted to hospitals, suffer from low overall life quality, and greatly reduced life expectancy, especially among children younger than five years old (Ware et al., 2017).

Access to care and treatment remains a significant challenge in Ghana. While hydroxyurea has been recognized as an effective therapy for reducing complications of SCD, its availability is inconsistent, and its cost is prohibitive for many families (Tshilolo et al., 2019). Blood transfusions, critical for managing severe anemia, are often limited by chronic shortages in the national blood supply (Grosse et al., 2011). Furthermore, the healthcare system faces vital gaps in infrastructure and expertise, particularly in rural areas where specialized care is almost nonexistent. Ghana has made progress in addressing SCD through pilot newborn screening programs in selected regions, leading to early detection and management in some areas (Ohene-Frempong et al., 2008). Public education campaigns and community-based initiatives have been launched to raise awareness, combat stigma, and promote genetic counseling. However, these programs have not been implemented at the scale necessary to address the country's SCD burden effectively (Ghana Ministry of Health, 2021).

Social and economic factors further exacerbate the challenges of SCD in Ghana. Many households struggle with the financial burden of frequent healthcare visits, medication

costs, and hospitalizations. Additionally, social stigma associated with genetic disorders like SCD negatively impacts affected individuals, reducing their access to healthcare and community support (Piel et al., 2017).Addressing the burden of SCD in Ghana requires coordinated efforts from all stakeholders. Expanding nationwide newborn screening programs can enhance early diagnosis and treatment outcomes. Improving access to hydroxyurea and ensuring sufficient blood supply could significantly improve survival rates and quality of life for SCD patients. Strengthening healthcare infrastructure, enhancing medical training, and fostering global partnerships are also essential to advancing SCD management. Finally, sustained public education campaigns are crucial for reducing stigma and promoting informed reproductive choices through genetic counseling (Piel et al., 2013).

Sickle Cell Diseases Prevalence in Zimbabwean

Sickle cell anemia is common among people from Sub-Saharan Africa, India, Saudi Arabia, and the Mediterranean area (Piel et al., 2017). The gene frequency rose as migrants transported more copies to the Americas, resulting in a more widespread worldwide distribution of the illness (Rees et al., 2010). In Sub-Saharan Africa, the disease affects about 2% of newborns, changing rates by area (Williams & Weatherall, 2012). A person with sickle cell trait (HbAS) only receives one parent's sickle cell gene and does not fully develop the illness. Less than 1% of people in South Africa have the sickle cell trait, but 10%–40% of people in equatorial Africa and 1%–2% of people along the coast of North Africa have it (WHO, 2020). In equatorial Africa, where malaria is prevalent, the highest incidence is noted. The gene's maintenance in afflicted groups has been facilitated by the selection advantage that the sickle cell trait provides against severe malaria (Allison, 1954).According to Ndeezi et al. (2016), sickle cell trait prevalence rates range from 15% to 30% in West African countries like Ghana and Nigeria, and up to 45% among the Baamba tribe in western Uganda. A person with sickle cell disease has the hereditary disorder for the rest of their life. Medical therapies can be used to control some of its symptoms, though. A stem cell transplant is the only known cure for sickle cell disease (SCD), and it has a success rate of about 85–90% in patients who qualify (Bernaudin et al., 2016). Unfortunately, access to such treatment is severely constrained in low-resource

countries, such as Zimbabwe, where it is virtually unavailable (Macani et al., 2011). Although newborn screening for SCD is critical for early diagnosis and therapy, most nations with the highest disease burden do not have broad screening programs in place (McGann, 2014). Due to this disparity, the precise number of children born with SCD in Zimbabwe and other resource-constrained countries is unclear. However, estimations based on carrier frequency and global birth rates indicate that about 312,000 children with SCD are born each year worldwide. This includes roughly 300 newborns in the UK and over 3,000 in the US (Piel et al., 2013).

Comparative Analysis of Sickle Cell Disease Distribution and Allele Frequencies in Zimbabwe and Sub-Saharan Africa

When comparing the distribution of sickle cell disease (SCD) genotypes and allele frequencies in African nations and Zimbabwe, several factors are taken into account, including malaria prevalence, genetic diversity, healthcare access, and historical consequences. The similarities, differences, and significant variables impacting these distributions give insight into the regional SCD epidemiology. Malaria has traditionally influenced the distribution of sickle cell alleles in malaria-endemic locations across Sub-Saharan Africa, including Zimbabwe (Allison 1954). The HbAS genotype (sickle cell trait) provides a selective advantage against malaria, leading to higher frequencies of the sickle cell allele in particular areas (Williams et al., 2005).Zimbabwe and West, Central, and East African countries (such as Nigeria, Ghana, and Kenya) have high rates of sickle cell trait (HbAS) due to its capacity to protect against Plasmodium falciparum malaria (Piel et al., 2013). In sub-Saharan Africa, the sickle cell trait (HbAS) is common; estimates range from 10 to 40 percent in different regions (Piel et al., 2013). Zimbabwe has a high proportion of HbAS carriers (15–25%), as is common in countries with long histories of malaria exposure. As with sickle cell disease (homozygous HbSS), the frequency of the sickle cell allele affects the incidence of the disease. According to reports, there are 1% to 2% of HbSS cases in Zimbabwe, which is comparable to rates in East and Central Africa (Makani et al., 2013). Geographical and malarial variables have a major role in the spread of SCD in Zimbabwe and other African countries. HbAS frequencies are somewhat higher in West African countries like Nigeria and Ghana due to higher rates of malaria

transmission, despite the fact that malaria is common in Zimbabwe (Piel et al., 2010). Conversely, the prevalence of sickle cell trait in Zimbabwe is somewhat lower than in West African regions with high malaria rates, but higher than in areas with temperate temperatures or reduced malaria transmission, such as parts of Southern Africa (Piel et al., 2010).Access to healthcare and awareness are also essential (Makani et al., 2013). Zimbabwe's healthcare system is struggling due to economic problems, which limits its capacity to screen for and treat sickle cell disease in comparison to countries with more robust healthcare systems. However, there are persistent disparities in SCD care across rural and urban areas despite initiatives to increase awareness and offer genetic counseling (Grosse et al., 2011). Population structure and migration trends also influence allele frequencies. Compared to Zimbabwe's relatively homogenous population, South Africa and Kenya have more genetically diverse populations, whose cosmopolitan urban areas allow for significant genetic mixing (Tishkoff et al., 2009). Tishkoff et al. (2009) suggest that the allele frequencies of the Ndebele and Shona populations in Zimbabwe may differ from those of more varied groupings in South Africa or Kenya, where interethnic marriages are more prevalent. National healthcare policy differences also affect sickle cell disease awareness and treatment. Ghana and Nigeria have implemented statewide screening programs and comprehensive public health measures to combat sickle cell disease. Zimbabwe's still-developing screening and early detection systems lead to disparities in sickness reporting and care, even if awareness is improving (Grosse et al., 2011). Several factors affect sickle cell disease frequency in Zimbabwe and throughout Africa. The existence of malaria has exerted considerable evolutionary pressure, and the HbAS genotype confers a survival advantage in areas where malaria is common (Kwiatkowski, 2005). Even while malaria is still on the rise in Zimbabwe, the disease is not as bad as it is in other parts of West Africa, which might be one factor contributing to the slightly lower incidence of sickle cell alleles. Additionally, founder effects and genetic drift may impact allele frequencies, particularly in small or isolated populations (Jallow et al., 2009). Jallow et al. (2009) state that allele frequency variations may differ from those of larger, more genetically varied populations in other African countries due to past population dynamics, such as those of the Ndebele and Shona ethnic groups in Zimbabwe. The outcomes of sickle cell disease (SCD) are impacted by access to medical care and

therapies. The better survival rates recorded by countries with more advanced healthcare systems and greater access to treatments like hydroxyurea or frequent blood transfusions may have an impact on the persistence of the sickle cell allele in the population (McGann et al., 2013). Despite Zimbabwe's achievements in controlling sickle cell disease, healthcare disparities and economic constraints, particularly in rural regions, still impact sickness outcomes (McGann et al., 2013). Cultural and social factors, such as marriage traditions and awareness of genetic diseases, also have an impact on the distribution of SCD. In Zimbabwe, inadequate availability of genetic counseling leads to high-risk sickle cell disease pairings. Even though awareness campaigns are growing in popularity, their effects are still not uniform, especially in rural areas (Dennis-Antwi et al., 2011).

CHAPTER VI

Conclusion and Recommendation

Introduction

A genetic disorder known as sickle cell disease (SCD) has been triggered by mutations in the gene *HBB*, which is accountable for generating the hemoglobin component. Parents may pass these mutations down to their children. It is estimated that between 300,000 and 400,000 babies are afflicted with the illness each year across the globe, with the majority of cases happening in sub-Saharan Africa. Kato et al. (2018). The sickness is a result of poor sanitation and hygiene practices. Hemoglobin. Hemoglobin molecules that have sickle globin mutant units built in can polymerize. Erythrocytes, mostly made up of sickle hemoglobin polymers, look like they have squiggly lines on them and are more likely to break down.

The SCD phenotype may also be altered by a range of other pathophysiological processes, including the activation of the immune system and vasoconstriction. SCD is distinguished from different types of illness by its phenotypic multifarious nature, a feature of the sickness. A single phenotype characterizes other forms of the disease. Stroke, acute chest syndrome, and acute pain episodes are some examples of common acute side effects that may occur suddenly. Chronic conditions, such as chronic kidney disease, have the potential to cause harm to every organ in the body at some point in time. The use of hydroxycarbamide, blood transfusions, and hemopoietic stem cell transplantation are some of the treatment options that have the potential to lower the severity of the disorder. The sooner a patient is diagnosed with a sickness, the higher the likelihood of surviving the illness. Some countries have implemented universal newborn screening programs even though doing so can be challenging in settings characterized by low income and a high incidence of infection. Sickle cell disease, most often abbreviated as sickle cell disease (SCD), is a multi-system condition that disproportionately impacts the health of African Americans living in the United States. It is also the kind of genetic sickness that occurs more often in some areas of the globe.

Conclusion

This research suggests that the prevalence of sickle cell disease in Zimbabwe is low compared to that in many other African and African nations. Multiple studies have shown that African countries, particularly those in sub-Saharan Africa, have high rates of SCD. According to estimates provided by Mudathir et al. (2019), between 50 and 60 percent of newborns in Sudan are diagnosed with SCD each year. The same author's study revealed that 3.5% of 480 individuals in Sudan tested positive for SCD. In Uganda, the frequency of the disease is as high as 45% among the Baamba tribe, located in the western area of the nation (Ademola, 2015).

Additionally, projections suggest that 150,000 babies are born with SCD each year. This absurd proportion is much too high compared with the SCD frequency in Zimbabwe. The high incidence that is seen in countries like Nigeria and Ghana may be attributed to ignorance as well as religious and ethnic issues, while in Zimbabwe, awareness has been developed chiefly via premarital counseling. According to this research, pupils from Zimbabwe who are now enrolled in schools in Cyprus have the genotype of the wild type (AA) and do not have the homozygous condition (SS).

The results of this research will help shed light on the fair distribution of sickle cell characteristics among Zimbabwean students currently enrolled in higher education in Northern Cyprus. In addition, the results of this research will raise awareness among other international students, urging them to follow the safety measures that Zimbabwean students have been taking when they return to their home countries.

Recommendations

1. Data harmonization with other facilities in Africa is needed to allow people to work together and compare data.

2. More research is necessary to better characterize the SCD genotype and phenotype, develop newborn screening, offer data for policymakers, and enable optimal health care planning for individuals with SCD in Zimbabwe. These studies are also required to establish screening for newborns.

3. Rising awareness about sickle cell disease in affected countries: many people in affected countries are not aware of sickle cell disease and its effects on them. Awareness campaigns should be conducted to educate people about the disease.

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Appendices

NEAR EAST UNIVERSITY
SCIENTIFIC RESEARCH ETHICS COMMITTEE

RESEARCH PROJECT EVALUATION REPORT

Meeting date :21.12.2023
Meeting Number :2023/119
Project number :1713

The project entitled **"The Ethnic Distribution of Sickle Cell Trait in Zimbabweans in Northern Cyprus"** (Project no: NEU/2023/119-1713), which will be conducted by Assoc. Prof. Dr. Mahmut Çerkez Ergören has been reviewed and approved by the Near East University Scientific Research Ethical Committee.

Prof. Dr. Şanda Çalı
Near East University
Head of Scientific Research Ethics Committee

Committee Member	*Decision*	*Meeting Attendance*
	Approved (✓) / Rejected (X)	*Attended (✓) / Not attended(X)*
Prof. Dr. Tamer Yılmaz	✓	✓
Prof. Dr. Şahan Saygı	✓	✓
Prof. Dr. İlker Etikan	✓	✓
Doç. Dr. Mehtap Tınazlı	✓	✓
Doç. Dr. Nilüfer Galip Çelik	X	X
Doç. Dr. Dilek Sarpkaya Güder	✓	✓
Doç. Dr. Gulifeiya Abuduxike	✓	✓
Doç. Dr. Burçin Şanlıdağ	✓	✓

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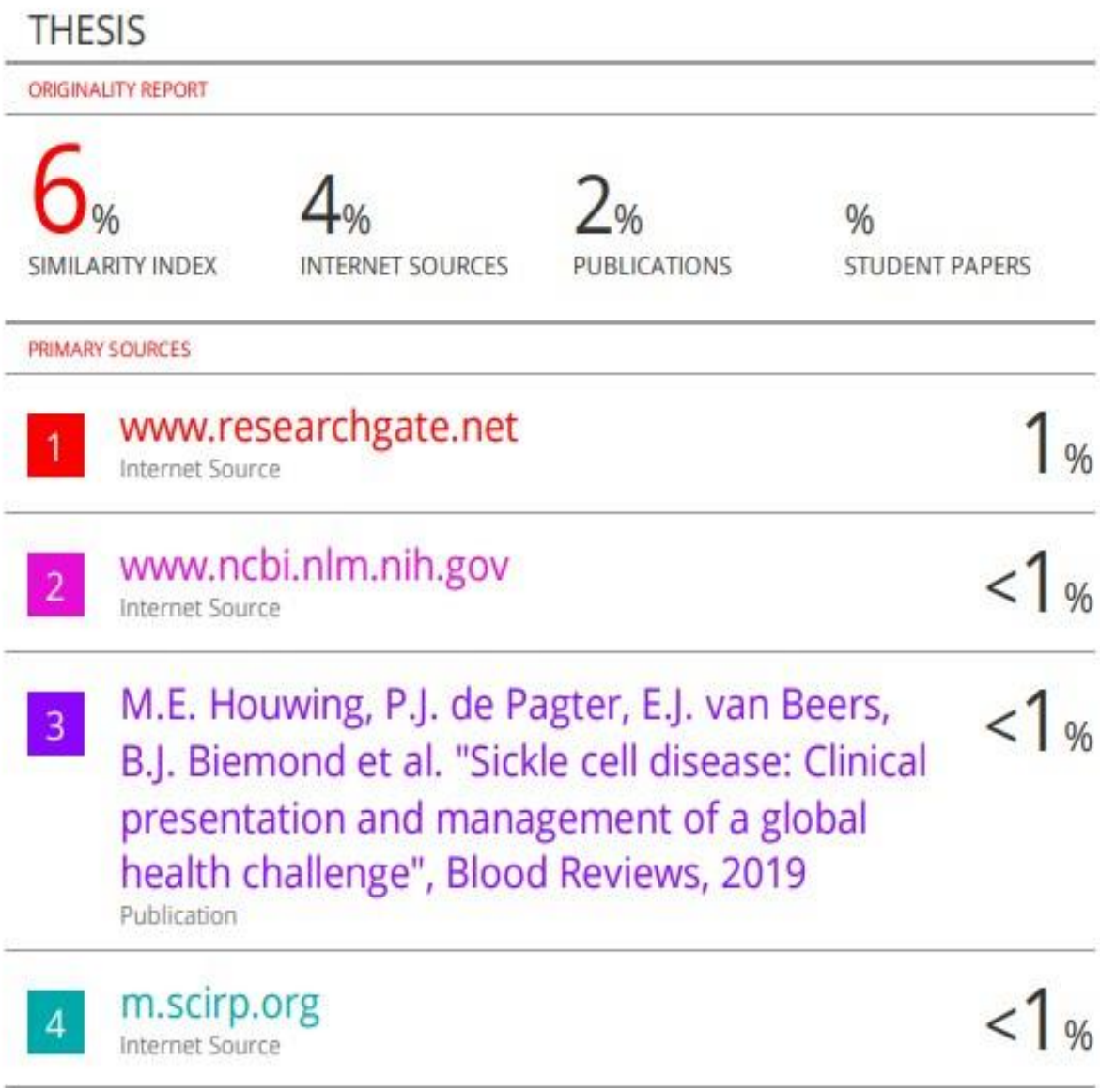

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