The Importance of Testing for Sickle Cell Anemia

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Abstracts

Sickle cell disease is a disease caused by a hemoglobin disorder inherited from parents, a mutated hemoglobin gene. Hemoglobin disorders such as thalassemia and blood cancer are seen all over the world. Approximately 5% of the world's population has genes that cause hemoglobinopathies. According to WHO 1% (on an average) population is born with the history of Sickle cell. The severity of the ailment surfaces at certain points of life cycle of the individual. The testing of the same is very important and then the respective curative measures are required to be taken. This present study will elaborate the reasons, testing and the importance of testing in developing countries with the help of previous studies conducted in this regard. Study is based on secondary data and present the cumulative outcomes thereof.

Keywords: Sickle cell Anemia, Testing, methods of testing, reasons.

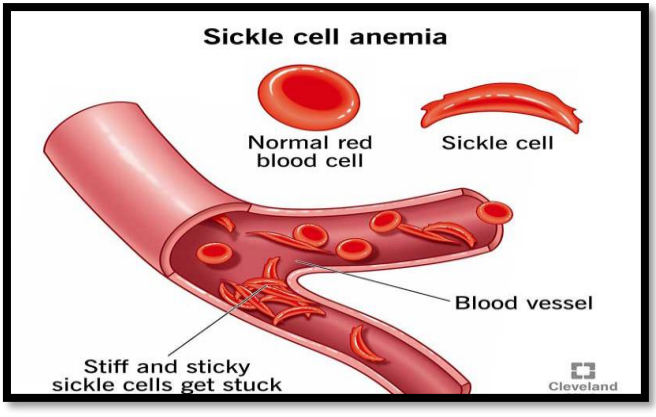
1. Introduction

Sickle cell disease (also known as sickle cell disease or sickle cell disease) is a disease caused by a hemoglobin disorder inherited from parents, a mutated hemoglobin gene. Hemoglobin disorders such as thalassemia and blood cancer are seen all over the world. Approximately 5% of the world's population has genes that cause hemoglobinopathies. Obeagu et al (2015) Approximately 300,000 babies are born with severe hemoglobin each year, including more than 200,000 diabetics in Saudi Arabia. Worldwide, there are more thalassemia carriers (i.e. healthy people who inherit the gene mutation from only one parent) than people with diabetes, but the high frequency of the sickle cell gene in some regions puts babies at higher risk of having . . . Migration is increasing the frequency of the disease in the Americas. In some parts of sub-Saharan Africa, up to 2 percent of children are born with the disease. Overall, the incidence of transgenics (healthy carriers of a genetic mutation from one parent) is 10 to 40 percent, with a

prevalence of 1 to 2 percent in Saudi Arabia. are falling ... This distribution reflects the fact that cancer contributes to immunity to malaria and that high malaria selection has led to a high frequency of gene mutations, especially in areas with high malaria, in the western part of the country. Obeagu (2020); Obeagu (2018)

The frequency of being a carrier determines the incidence of sickle cell disease at birth. For example, in Qatar, the most populous country in the subregion, 24% of the population is transgender and the incidence of sickle cell disease is around 20 per 1,000 births. This means that 150,000 children are born with leukemia each year. Some malaria antibodies tend to survive and then spread via the abnormal hemoglobin gene. While one variant protects against malaria, inheriting two variants that cause leukemia and malaria is the cause of the disease. Poor hygiene and death in children with diabetes. There is growing evidence that malaria in the country not only affects outcomes, but also changes the appearance of sickle cell disease. Rees (2010)

The public health impact of sickle cell disease is significant. Its impact on human health can be measured using a sample of infant and under-five mortality. Since not all deaths occur in the first year of life, the most important measure is the mortality of children under five. Today, more affected children survive past the age of five but are still at risk of premature death. When measuring its health impact in terms of under-five mortality, sickle cell disease accounts for 2% of under-five mortality in the country. Bunu (2023) In 1994, life expectancy for men in the United States was estimated at 42 years and for women at 48 years, while similar data published in Saudi Arabia in 2001 showed life expectancy for men as 53 years and for women as 58.5 years. The mortality rate is higher in urban areas of the country than in some regions, and estimates based on the age of those presenting to outpatient clinics show that half of the patients are elderly. Diabetes causes death up to the age of five, mostly with red blood cell deaths due to malaria and pneumococcal sepsis. Njar (2020)



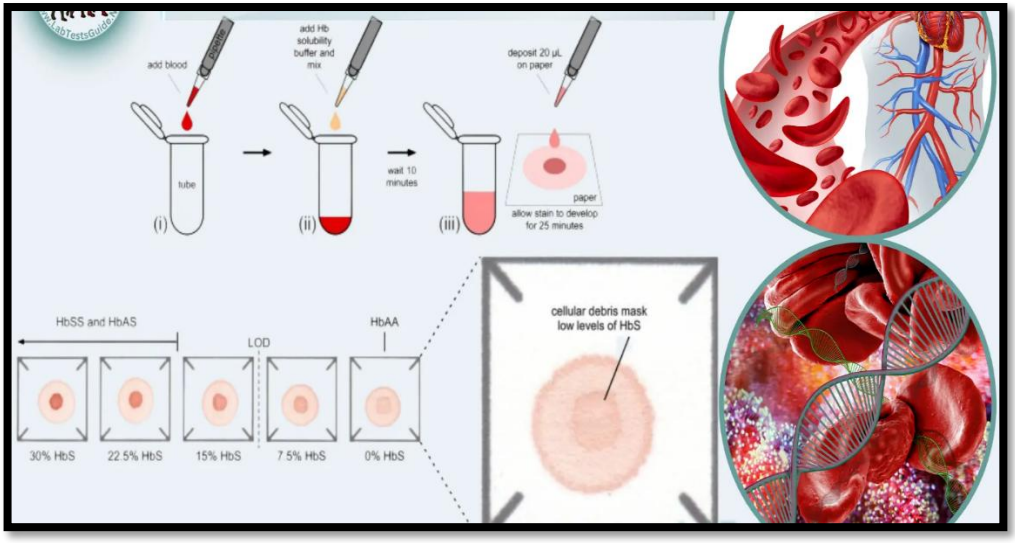
Source: https://my.clevelandclinic.org/health/diseases/4579-sickle-cell-anemia

Figure 1: Sickle Cell Anemia

2. Basic Characteristics

Sickle cell disease covers a wide range of diseases. Most patients have diabetes with a hemoglobin concentration of about 8 g/dl. The main problem is that red blood cells tend to become inflamed and block capillaries when oxygen pressure is low. In children, red blood cells are often produced in the spleen, which leads to a high risk of death before the age of seven from rapidly progressive anemia due to the development of the spleen or from serious infection due to the absence of the spleen. Children between the ages of 6 and 18 months often have swelling of the hands and/or feet (hand-foot syndrome). Swem (2018) Survivors may also suffer from recurrent, undiagnosed complications such as "chest pain" (pneumonia or pulmonary infarction), bone or joint pain, priapism, or non-functioning kidney disease. For most patients, the risk of complications can be reduced by simple measures such as using penicillin in childhood, avoiding burns, cold, and dehydration, and contacting a specialist center early. These measures are most effective if the baby is diagnosed at birth. Some patients' problems are so severe that they require regular blood transfusions and iron chelation therapy. This, together with the development of diabetes in Saudi Arabia, creates an urgent need to develop appropriate standards of care for the management of the disease in the country. Obeagu et al (2023)

Diabetes is a serious public health problem, its management is still inadequate, national management systems are not in place, easy facilities for patient management are often not available, screening is rarely done and most patients are diagnosed when there is a major problem. Simple, cheap and effective methods such as penicillin to prevent infections are not available in many countries. An important aspect of patient care is early intervention with preventive measures such as medication, antibiotics, nutrition, folic acid supplementation and drinking plenty of water. Hydroxyurea treatment reduces many serious problems. There is evidence that screening for diabetes in newborns, when combined with timely diagnosis, parent education and quality care, can reduce the morbidity and mortality of this disease in infants and young children. Even the best care, including expert consultation and access to necessary care, can reduce morbidity and mortality and improve the quality of life of patients with diabetes in developing countries, regardless of the patient's ability to pay. In the past decade, many advances have been made: Long-term treatment with hydroxyurea has reduced the incidence of severe pain and improved the quality of life of patients with diabetes; it has helped control life-related diseases such as stroke and chest pain; efficacy was achieved in animal models in 2016 but has not yet been tested in human clinical trials. Unfortunately, these advances, which have been implemented mostly in wealthy countries, have widened the gap between the quality of life of patients in developed and developing countries, and this gap can only be closed by improving health. Adegoke (2015)



Source: https://www.labtestsguide.com/sickle-cell-screening-assay

Figure 2: Sickle Cell Screening

3. Testing Methods

I. Conventional Methods

1. Screening tests

a. Sickling test:

Diagnosis is usually based on polymerization of HbS in the deoxygenated state. The most commonly used method today is the solubility test of its components, which is based on the insolubility of Hb-S in the presence of concentrated phosphate buffers, hemolytic reagents, and sodium dithionate. The reagents crystallize HbS and precipitate cells, refracting light and causing the solution to become cloudy. Compare the results of negative and positive controls. Makani (2007)

b. Solubility test:

This technology is based on the properties of the substrate and the insolubility of Hb S, allowing the interpretation to detect the presence of heme S. The mixture is then placed on chromatography paper and stained. Differential blood samples are made from hemoglobin and these stains are used to determine Hb SS, carrier Hb AS and normal hemoglobin Hb AA. This test showed 94.2% sensitivity and 97.7% specificity for the detection of Hb S. Hb SS produces a reddish residue and a clear filtrate. Piel et al (2013)

2. Full Blood Count (FBC):

Complete blood count (FBC) is the initial diagnostic test to diagnose various types of diabetes. However, hematological parameters may be affected, suggesting mutations. Edward et al (2022) Patients with sickle cell anaemia usually present with the following result

- Anaemia
- Neutrophilia
- Thrombocytosis
- Elevated Mean corpuscular volume (MCV)
- Elevated Red cell distribution width (RDW)

3. Peripheral Blood film:

Peripheral blood smear (PBF) is usually performed after an abnormal automatic count is detected and is considered an important part of the hematologic evaluation. PBF examines the morphology of blood cells and evaluates changes that can provide important information to help identify different types of diabetes. Blood film analysis is very difficult due to changes in cell edges, position, shape, and size. Obeagu (2018)

In sickle cell anemia the following features are seen

- Normocytic normochromic
- Irreversible Sickle Cells (ISCs)
- Nucleated Red Blood Cells (NRBCs)
- Target cells
- Neutrophilia
- Thrombocytosis

4. Haemoglobin electrophoresis –

Electrophoresis is a type of chromatographic technique and is considered one of the important tests to test Hb variations. In this experiment, an electric current is used to promote the passage of electronic components. The first use of electrophoresis to identify the hemoglobin variant Hb-S occurred in 1949. Telfer et al (2007)

Some Advanced Methods

Prenatal testing

a. Amniocentesis: Amniocentesis is a test done during pregnancy to check if there are genetic or chromosomal problems in the baby, such as diabetes, thalassemia, Down syndrome. in the middle, but can be done later if necessary. It can be done earlier, but is usually avoided because it increases the risk of amniocentesis complications. During the examination, a long,

thin needle is inserted through the abdominal wall, guided by ultrasound images. A needle is inserted into the amniotic sac surrounding the baby, and a small sample of amniotic fluid is taken for analysis. The test itself usually takes about 10 minutes, but the entire session takes about 30 minutes. The test results will show if your child has a genetic predisposition to leukemia. Telfer et al (2018)

b. Chorionic Villus Sampling: This can be done safely during pregnancy (about 9 weeks after your last menstrual period). The best time is 10 to 14 weeks. A small amount of material is taken from the placenta, which is made up of tissue from the baby, not the mother, so it shares the same genetic material. It is made up of chorionic villi. Villi is the plural of villi. There are two ways to get a chorionic villus sample, either from the stomach or the cervix. For this reason, most samples are taken from the abdomen, where the chance of pregnancy is lower than samples taken from the vagina and uterus. Nnodim et al (2015) An ultrasound scan is used to help us see what we are doing. Before inserting the needle, a local anesthetic is injected into the skin to numb the pain. Instead, the obstetrician passes very thin forceps through the vagina and into the uterus. It is so thin that most women don't really need it. It can't cope with the baby or the water bags around the baby. The forceps are opened and closed to remove the small chorionic villus sample from the placenta. After the sample is taken, it is examined under a microscope immediately to make sure it is a suitable sample from the placenta. If so, we stop. If it's not the right pattern, we move the cap of the tube or the needle slightly to get the right pattern. The test usually takes 10 to 20 minutes. Researchers look at the DNA of the chorionic villus to determine if the child has the heme gene or if there is a mutation from the parent. Adewoyin (2015)

High Performance Liquid Chromatography (HPLC):

HPLC has been used to separate hemoglobin due to differences in the stationary phase. HPLC detects different types of heme depending on the retention time and image pressure. Each hemoglobin has a specific retention time that can be compared to the retention time of known hemoglobin products. The development of fully automated HPLC will allow accurate analysis of large samples. HPLC is more effective than electrophoresis in separating hemoglobin variants. HPLC is less sensitive and more reliable in monitoring patients receiving serum or hydroxyurea. However, HPLC is an expensive machine and cannot distinguish between all variants with the same retention time. For example, all different Hb concentrations with similar retention times overlap the Hb S peak. Therefore, it will misdiagnose new variants that mimic Hb S. Aloh (2015)

Genetic analyses:

a. Polymerase Chain Reaction (PCR)-

Polymerase chain reaction is one of the most powerful diagnostic tests, using specific enzymes along with specific primers to amplify a portion of genetic material into millions of copies. PCR can detect a single or multiple known genes in a single tube. The PCR process involves repeated denaturation, annealing, and extension for 20-40 thermal cycles. Results can be detected by gel electrophoresis, pooling, measuring the curve, or monitoring changes in fluorescence. The sensitivity and specificity of PCR have revolutionized prenatal and childhood diagnosis. Various PCR-based methods for the detection of β-mutations have been documented, such as the high melting point test (HRM), which is simple, sensitive, and cost-effective and can be used for

clinical size analysis of SCD genotypes. Another simple, low-cost PCR-based technology was developed using bidirectional allele-specific amplification (ASA) and the HotStar system to provide more specific single-tube genotyping of sickle cell anemia. Point mutations were used to make SNP samples. In addition, discriminatory conditions lead to the determination of homozygous and heterozygous states based on differences in size on agarose gel electrophoresis. Chibunna et al (2015)

b. Restriction Fragment Length Polymorphism:

Restriction fragment length polymorphism (RFLP) is used to identify cell lines based on restriction enzymes that eliminate the recognition site of the βs mutant gene. For example, MstII was one of the first restriction enzymes described; therefore, when thymine replaces adenine, it eliminates the recognition site of the MstII restriction enzyme. The number of bands formed by enzyme digestion after separation indicates the number of mutations. In healthy individuals with (βA βA), the gene is cleaved by the MstII restriction enzyme and forms two groups. In homozygotes, the restriction enzyme cuts both genes, resulting in two short genes. In the tumorigenic (βAβS) gene, there is no break at βS and one cluster results, but when the βA gene is broken, two clusters result. In homozygotes for type 2 diabetes (βSβS), there is no enzymatic cleavage due to mutations in both genes, resulting in a single gene mutation. Another restriction enzyme used in cell culture is Ddel I. Therefore, bands of different lengths appear depending on the presence of the sickle cell mutation. Kanter et al. demonstrate a platform known as Sickle SCAN. used to diagnose heme Hb AA, sick cell Hb AS, heme C trait Hb AC, sick heme C sick Hb SC and heme C sick Hb CC. This test uses lateral flow immunoassay polyclonal antibodies against heme S, heme C and heme A to detect different types of SCD. Polyclonal antibodies bound to the test strip; Hemoglobin binds to the corresponding antibodies and produces a blue line. The Sickle SCAN box contains four test strips: control strip, normal Hb A, Hb S strip and Hb C strip. The test can be performed in a few minutes and costs a few dollars per test. McGann et al. The performance of Sickle SCAN in the detection of different heme species was tested. This experiment was performed using 139 whole blood samples (venous samples, dried blood and serum samples) and the results were compared with capillary zone electrophoresis (CZE). The additional sensitivity and specificity of this test for Hb SS are 98.4% and 98.6%, respectively. The additional sensitivity and specificity for the diagnosis of Hb SC are 100%. Analysis of infant samples with high Hb F showed that the detection of Hb S or Hb C was not affected by the elevation of HbF. They also checked the storage location of the assay and noted that the device can be stored for 30 days at 37°C. However, this assay has some limitations, such as misinterpretation of results due to visual readings, interactions between polyclonal antibodies, and false positives when Hb A versus Hb S heterozygotes are detected. The examination is performed in PHCs.

4. Conclusion

Understanding the latest advances in the diagnosis and treatment of stem cell anemia is an important way to improve the lives of people affected by this disease. It empowers patients, their families and their doctors to make informed decisions, access the most advanced treatments and

advocate for better care. Thanks to ongoing research and exciting work, there is real hope for a better future for people living with leukemia. Together, we can contribute to a future where people with this condition can not only manage their health, but thrive with the new energy and strength that comes from collaboration and work.

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