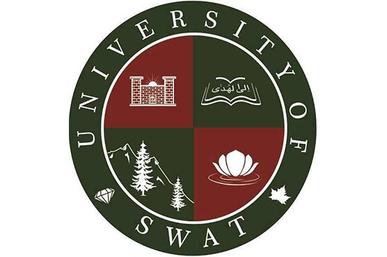
**TO STUDY THE MUTATIONS IN HBB GENE OF SICKLE CELL DISEASES AND TO CORRELATE THESE MUTATIONS WIH THE DISEASE SEVERITY IN THE DISTRICT SWAT**

****

**MENA IBRAHIM**

**MPHILL ZOOLOGY**

**INTRODUCTION**

Sickle cell diseases are inherited disorders. In these disorders the genes encoding the beta subunits of hemoglobin are mutated. (Raees *et al*., 2010) Genes present on chromosomes 11 and 16 are responsible for the synthesis of hemoglobin. Thalassemia and sickle cell diseases are caused by disorder in hemoglobin. They are included in the list of firstly identified molecular diseases. (Rund and Rachmilewitz, 2005) Sickle cell diseases are structural disease of hemoglobin which actually distorted the structure of red blood cells while thalassemia is a hemoglobin synthesis disorder. (Rund and Rachmilewitz, 2005) Normally red blood cells are elastic having the shape of biconcave disc so they can easily pass through the capillaries. (Capriotti et al., 2016) The mutant genotypes of hemoglobin causes the red blood cells to become sickle shaped. (Steinberg and Sebastiani, 2012)

SCA (sickle cell anemia) is one genotype while Hemoglobin SC (HbSC) is another genotype of SCD (sickle cell disease). HbSC, individuals inherit HbS (sickle hemoglobin) in association with hemoglobin C (HbC). (Serjeant *et al* 2018) According to the book ‘’The Disease of Tropical Climates and their Treatment (1872),by Dr. Africanus Horton he gave the first description of a disorder "like" sickle cell anemia (SCA). But it wasn't clear until 1910 when Drs. James B. Herrick and Ernest Irons noted in a dental student "sickle-shaped" red cells. (Rees et al 2010) Neel found that a single change in the DNA sequence of a gene that makes hemoglobin can cause the production of abnormal hemoglobin, which is called sickle cell hemoglobin. (Neel J. V., 1949)

Around five to six months of age is the normal onset of issues with sickle cell disease. Anemia, swelling in the hands and feet, infections caused by different bacteria, stroke, and body pain episodes are a few health issues that could arise. (National Heart, Lung, and Blood Institute, 2017). Pain episodes also known as sickle cell crises and organ damage are common in sickle cell anemic patients. (Kato et al., 2018) Vaso obstruction is originated by the sickle cells in the sickle cell anemic patients because they are less flexible and form polymers which obstruct the blood vessel.  (Kato et al., 2018)

In the HbSC genotype, the number of RBCs and the amount of Hb in the blood are increased. (nagel et al 2003) ( Aleluia et al 2017) Altered, K–Cl co transporter in HbSC disease is the cause of dehydration of RBC, which makes the concentration, of intracellular hemoglobin high due to which it become denser than HbAA-containing RBC. (nagel et al., 2003)

Sickle cell diseases that are HbSC disease and SCA disease share the same molecular basis but they just differ in point mutation; the single nitrogenous base mutation is GAG to AAG, in which the glutamic acid is replaced by lysine, in the globins chain. (Serjeant *et al* 2018). The clinical episodes are typically more common in sickle cell anemia (SCA) patients compared to hemoglobin SC (HbSC) illness, which is regarded as a less severe kind of sickle cell diseases (SCD). (Aleluia et al., 2017) (Kato et al., 2018)

To find the gene mutation in Sickle cell diseases, gene sequencing is the specific and accurate method and specially next generation sequencing is better than maxam gillbart as takes less time and the results are more authentic and is fast process.DNA extraction using pci method is time taking but more precise than other methods and PCR is the fastest technique for making copies of DNA segments.

These techniques are affordable at the University of Swat. Using questionnaires in this method is also more reliable approach towards the patients than clinical observations and oral questions. Blood samples would be collected using EDTA tubes to extract DNA and to visualize sickle cells as they are sickle cell disorders of blood.

Blood related disorders have become major health concern in Pakistan. These health problems include the sickle cell disease and its other mutations like HBC and HBD Punjab. On the other hand thalassemia has also increased to some extent it may be thalassemia major or minor. (Ghani et al., 2002) People from the Kharadar, Lyari, Korangi, and Malir localities of Karachi were at a higher risk of health problems the contributing factors are their movement from place to place (migration), family marriages, financial status, social or cultural status, and knowledge or educational background. These people are actually sharing low socioeconomic status. (Ghani et al., 2002)

A study in Brazil found that people with sickle cell anemia (SCA) had the most severe anemia, hemolysis, and increased white blood cell counts. They also had high levels of inflammatory substances in their blood. On the other hand, people with HbSC had higher levels of fats and renal markers in their blood. (da Guarda et al., 2020)**.** In developed societies, people with sickle cell disease (SS) now have a median survival of 45 to 55 years, which is similar to the general population. This is due to the upgraded techniques in health care, such as advanced diagnostic tools and treatment, and access to pain management and other supportive care. (Platt et al. 1994; Wierenga et al. 2001).

The existing knowledge has given us many ideas about the mutation but As far as I found that it is necessary to work at this topic at this particular area because the survival rates of the patients are very less. The mutations are not clearly identified and this is why the symptoms are getting severe and severe due to the lack of proper treatments.

Sickle cell anemia is actually a lifelong hemolytic **anemia** which requires blood transfusions. (Kato et al., 2018) Patients with sickle cell disease can get an immunization and chemotherapy to avoid infections. In addition to pain relievers and folic acid medications, substantial fluid consumption is recommended. (World Health Organization, 2011)

The study area of this research work would be the district swat. Blood samples will be collected from the diseased patients. Through blood smears the sickle cell can be visualized and observed under the microscope. After that the DNA would be extracted using PCI methods which time is taking but accurate method. The extracted DNA will be then placed in the PCR using PCR mixtures which will make multiple copies of the genes which will be run over the gel using agarose gel electrophoresis, the gel will be then observed under gel doc for mutations. Using the next generation sequencing the exact mutated nitrogenous bases can be observed.

This researchstudy would help us to find out the disease severity of the mutations in the specific population. This will be used in the prediction of proper medications. New treatments can be established using this knowledge through which the disease symptoms can be controlled. Different managements could be done by which the survival rates can be increased.

**RESEARCH QUESTION**

What is the effect of different mutations on the disease severity? If the mutation is changing so it is necessary that symptoms will also change?

**AIMS AND OBJECTIVES**

1. I will prepare and observe the blood smears under microscope to visualize sickle cells.
2. The aim of my study will be to find the mutations in HBB gene in sickle cell anemia.
3. The mutations will also be compared with the disease severity.

**MATERIALS AND METHODS**

**EXCLUSION AND INCLUSION CRITERIA**

Only the SCA ,HBSC and sickle cell trait mutations will be included and all other mutations will be excluded.

**ETHICAL APPROVAL**

* For the progress of my research work I will need ethical approval from the ethical approval authority.

**STUDY AREA**

* The study area of my research work will be the district swat.

**SAMPLES COLLECTION**

Samples will be in the form of blood. Blood will be collected from sickle cell anemic patients

* I will collect the blood from the clean and dry area.
* The venipuncture site will be cleaned with an antiseptic wipe.
* The needle will be inserted into the vein carefully.
* To prevent hemolysis blood will be collected slowly.
* After collection the blood will be capped immediately.
* Lavender top tube: an anticoagulant called potassium EDTA, is present in this tube which prevents the blood from clotting.

**BLOOD SMEAR FORMATION**

* Blood smear will be formed on the slide and will be observed under the microscope

**DNA EXTRACTION**

* Dna will be extracted using pci method. PCI stands for phenol chloroform isoamyl alcohol. It works on the principle of liquid- liquid extraction of biomolecules.
* First the blood sample is mixed with phenol and chloroform solution. Proteins are denatured and are dissolved in phenol.
* After this the centrifugation process is done which separates aqueous phase and organic phase.dna is present in the aqueous phase.
* Emulsification is prevented by isoamyl alcohol.
* The aqueous phase is collected and ethanol is added to precipitate the dna.

**POLYMERASE CHAIN REACTION**

**PCR REQUIREMENTS**

* DNA template
* DNA primers
* DNA polymerase
* PCR buffer
* Magnesium chloride (MgCl2): This is a cofactor for DNA polymerase
* dNTPs
* Thermal cycler

**PCR STEPS**

* The DNA template is denatured (broken into two single strands) by heating the PCR reaction mixture to a high temperature (95°C).
* To enable the DNA primers to attach to the ends of the single-stranded DNA templates, the temperature is then decreased (to 50–65°C).
* In order for the DNA polymerase to create new DNA strands by extending the primers from the ends of the DNA templates, the temperature is then raised once more (to 72°C).
* 20–40 times, doubling the number of DNA copies each time, steps 2 and 3 are repeated.
* The PCR process is subsequently stopped by bringing the mixture's temperature down to 4°C.

**GEL ELETROPHORESIS**

* . A polymer with small pores, such agarose or polyacrylamide, is used to create the gel. The size of the DNA fragments that can be separated depends on the size of the pores.
* The gel will be added into the tray. At one end of the gel, the wells will be filled with the DNA samples.
* The gel will be subjected to an electric field, which will cause the DNA fragments to migrate in the direction of the positive electrode.
* The DNA fragments allowed splitting on the gel by running it for a while.
* A dye will be use to color the gel, which will bond to the DNA fragments and make them visible.
* DNA pieces will be visualized Using del doc.

**DNA SEQUENCING**

Next-generation sequencing is a set of DNA sequencing technologies that can quickly sequence many DNA fragments at the same time.

The most popular NGS platform is Illumina, according to research. It is based on sequencing by synthesis (SBS), a method for sequencing DNA that makes use of fluorescently tagged nucleotides. So i will perform illumina next generation sequencing method.

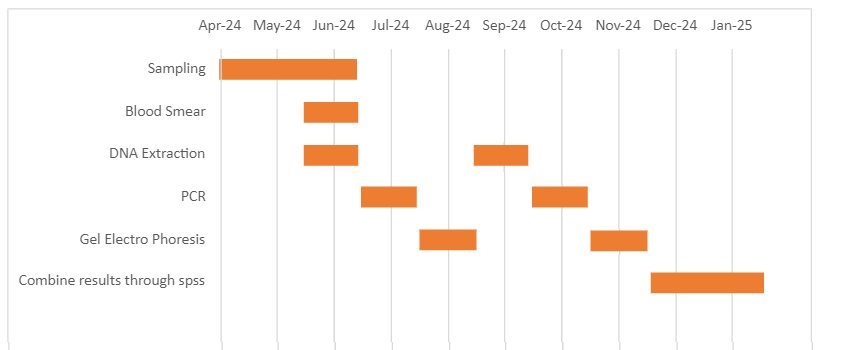
**SPSS (STATISTICAL PACKAGES OF SOCIAL SCIENCES)**

For further data analysis SPSS software will be used which perform different correlation tests.

Questionnaires will be distributed among the patients and the through which family history and symptoms severity can be checked which will be further processed in the SPSS.

**GANTT CHART**

I will start my research on December 2024 and will conclude in January 2025. It will take 10 to 11 months.



**BUDGET CHART**

The total cost on my research work would be 298,200.00 PKR.

**REFERENCES**

Aleluia, M.M., Fonseca, T.C.C., Souza, R.Q., Neves, F.I., da Guarda, C.C., Santiago, R.P., Cunha, B.L.A., Figueiredo, C.V.B., Santana, S.S., da Paz, S.S., Ferreira, J.R.D., Cerqueira, B.A.V., Gonçalves, M. de S., 2017. Comparative study of sickle cell anemia and hemoglobin SC disease: clinical characterization, laboratory biomarkers and genetic profiles. BMC Hematol. 17, 1–10. https://doi.org/10.1186/s12878-017-0087-7

Jones, S., Duncan, E.R., Thomas, N., Walters, J., Dick, M.C., Height, S.E., Stephens, A.D., Thein, S.L., Rees, D.C., 2005. Windy weather and low humidity are associated with an increased number of hospital admissions for acute pain and sickle cell disease in an urban environment with a maritime temperate climate. Br. J. Haematol. 131, 530–533. https://doi.org/10.1111/j.1365-2141.2005.05799.x

Nagel, R.L., Fabry, M.E., Steinberg, M.H., 2003. The paradox of hemoglobin SC disease. Blood Rev. 17, 167–178. https://doi.org/10.1016/S0268-960X(03)00003-1

National Heart, Institute, B., 2017. What are the signs and symptoms of sickle cell disease.

Neel, J.V., 1949. The Inheritance of Sickle Cell Anemia. Science 110, 64–66. https://doi.org/10.1126/science.110.2846.64

Platt, O.S., Brambilla, D.J., Rosse, W.F., Milner, P.F., Castro, O., Steinberg, M.H., Klug, P.P., 1994. Mortality In Sickle Cell Disease – Life Expectancy and Risk Factors for Early Death. N. Engl. J. Med. 330, 1639–1644. https://doi.org/10.1056/NEJM199406093302303

Rees, D.C., Williams, T.N., Gladwin, M.T., 2010. Sickle-cell disease. The Lancet 376, 2018–2031. https://doi.org/10.1016/S0140-6736(10)61029-X

Serjeant, G.R., Vichinsky, E., 2018. Variability of homozygous sickle cell disease: The role of alpha and beta globin chain variation and other factors. Blood Cells. Mol. Dis., SI: Globins 70, 66–77. https://doi.org/10.1016/j.bcmd.2017.06.004

Steinberg, M.H., Sebastiani, P., 2012. Genetic Modifiers of Sickle Cell Disease. Am. J. Hematol. 87, 795–803. https://doi.org/10.1002/ajh.23232

Tewari, S., Brousse, V., Piel, F.B., Menzel, S., Rees, D.C., 2015. Environmental determinants of severity in sickle cell disease. Haematologica 100, 1108–1116. https://doi.org/10.3324/haematol.2014.120030

Kato, G.J., Piel, F.B., Reid, C.D., Gaston, M.H., Ohene-Frempong, K., Krishnamurti, L., Smith, W.R., Panepinto, J.A., Weatherall, D.J., Costa, F.F., Vichinsky, E.P., 2018. Sickle cell disease. Nat. Rev. Dis. Primer 4, 18010. https://doi.org/10.1038/nrdp.2018.10

Sickle Cell Inheritance [WWW Document], n.d. . Spark Sick. Cell Change. URL https://www.sparksicklecellchange.com/sickle-cell-genetics/inheritance (accessed 9.5.23).

Capriotti T, Frizzell JP (2016). Pathophysiology : introductory concepts and clinical perspectives. Philadelphia. [*ISBN*](https://en.wikipedia.org/wiki/ISBN_(identifier)) [*9780803615717*](https://en.wikipedia.org/wiki/Special:BookSources/9780803615717). [*OCLC*](https://en.wikipedia.org/wiki/OCLC_(identifier)) [*900626405*](https://www.worldcat.org/oclc/900626405).

["Sickle Cell Disease"](https://rarediseases.org/rare-diseases/sickle-cell-disease/). *NORD (National Organization for Rare Disorders)*. Retrieved 10 June 2019.

World Health Organization. (2011). Sickle-cell disease and other haemoglobin disorders. Fact Sheet No. 308. *World Health Organization, Media Centre January*, 1-3.

National Heart, Lung, and Blood Institute. (2017). What are the signs and symptoms of sickle cell disease.

World Health Organization. (2011). Sickle-cell disease and other haemoglobin disorders. Fact Sheet No. 308. *World Health Organization, Media Centre January*, 1-3