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Research Article

Effect of Glucose 6 Phosphate Dehydrogenase In Sickle Cell Anemia In Coastal Andhra Pradesh

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ABSTRACT

Objective:
To estimate the levels of Glucose 6 Phosphate Dehydrogenase and molecular analysis of Genetic variability in Sickle Cell Anemia, by PCR in tribal population of Coastal Andhra Pradesh.

Methods:
Microscopy and estimation of hemoglobin by hemoglobinocyanide method are the tests usually carried out to diagnose Sickle Cell Anemia. Apart from these primary tests, hemoglobin electrophoresis is also a characteristic diagnostic tool which is used to check different types of hemoglobin in blood. This separates the normal and abnormal types of Hb. Mainly PCR is the technology used to detect sickle cell anemic patients and heterozygous carrier. It synthesizes million copies of desired abnormal gene.

Results:
All the 30 patients under the present study were tested positive to Sickle Cell Anemia by carrying out microscopy, hemoglobinocyanide method, hemoglobin electrophoresis. The blood samples deoxygenated with Sodium Meta Bisulfite Solution, which leads to the sickling of the cells. The change in the structure of RBC can be visualized under the microscope. The microscopy confirms the presence of sickle cell trait, but it cannot specifically confirm the presence of disease. Hemoglobin electrophoresis is carried out, which is helpful in differentiating the types of hemoglobin in the blood. Electrophoresis separated normal and abnormal types of Hb. Citrate agar gel Electrophoresis was preferred here. Each Hb have different charges and different electrophoretic mobility and accordingly bands were formed. By Hemoglobinocyanide method, the anemic conditions in blood sample was detected. This was measured by using a colorimeter at 530nm. PCR is the rapid procedure for the diagnosis of Sickle Cell Anemia. In PCR, the specific forward and reverse primers sequence sets which can recognize and amplify Hb A, Hb S, Hb c genes. When the amplified gene sequences are subjected to electrophoresis, the Hb bands are observed. As this primer sequences can recognize homozygous and heterozygous types of SCA, the Hb SS and Hb SC bands are observed when placed in a Gel Doc. The Hb SS bands are formed at lower side of the gel compared to normal and Sc indicating the low amount of DNA in the sample.

Conclusion:
The present study establishes that Microscopy is the traditional method of diagnosis of Sickle Cell Anemia. It is the basic test identifies the presence of sickle cell trait, but it is unable to confirm the presence of disease. PCR is an advanced technique which was used specifically in detecting the abnormal Hb levels of both homozygous and heterozygous trait conditions. It specifies which gene is responsible in causing SC.

INTRODUCTION:

Sickle-cell disease (SCD), also known as sickle-cell anaemia (SCA), is a group of genetically passed down blood disorders. It results in an abnormality in the oxygen-carrying protein haemoglobin found in red blood cells. This leads to a rigid, sickle-like shape under certain circumstances. Problems in sickle cell disease typically begin around 5 to 6 months of age. A number of health problems may develop, such as attacks of pain ("sickle-cell crisis"), anemia, bacterial infections, and stroke. Long term pain may develop as people get older. The average life expectancy in the developed world is 50 years. Sickle-cell disease occurs when a person inherits two abnormal copies of the hemoglobin gene, one from each parent. Several subtypes exist, depending on the exact mutation in each hemoglobin gene. The sickle gene is an example of balanced polymorphism. Heterozygotes have a selective advantage and are protected against *Plasmodium falciparum* malaria while there is an increased premature death rate of homozygote Allele responsible for causing SCA is present on short arm of chromosome 11[9]. It leads to abnormal, rigid and sickle shaped RBC It is an autosomal recessive disorder is caused by mis substitution of valine instead of glutamic acid[1,2].Mutation turns Hb A TO Hb S GAG TO GTG (Transversion). It is an example of single nucleotide polymorphism (SNP). An attack can be set off by temperature changes, stress, dehydration, and high altitude. A person with a single abnormal copy does not usually have symptoms and is said to have sickle-cell trait. Such people are also referred to as carriers. Diagnosis is by a blood test and some countries test all babies at birth for the disease. Testing is also possible during pregnancy.

Sickled cells has low elasticity due to reduced oxygen tension, leads to Ischemia. It is the inheritance of the sickle genes that causes red blood cell (RBC) abnormality. All complications of Sickle Cell Disease can he traced to changes in the make-up of the RBC. Normal RBC's are smooth surfaced, enabling them to change their shape to flow through small blood vessels. Under certain conditions (i.e., acidosis, dehydration, infection, and low oxygen. etc.) RBC's containing Sickle Hemoglobin become rigid, elongated, and sickle shaped. Some RBCs sickle immediately, while others remain normal for hours before sickling. Most RBCs containing Sickle Hemoglobin can sickle and then unsickle. After repeated cycles of sickling and unsickling, the RBC's become irreversibly sickled.

SCA has genetic resistance to malaria as the defective Hb ruptures prematurely, hence the plasmodium is unable to affect RBC. The complications of sickle-cell disease can be managed to a large extent with vaccination, preventive antibiotics, high fluid intake, folic acid supplementation, and pain medication. Other measures may include blood transfusion, and the medication hydroxycarbamide (hydroxyurea). A small proportion of people can be cured by a transplant of bone marrow cells. G6PD deficiency is prevalent in many tribal groups in India[3]. A cross-section of 15 major tribal communities from different parts of Odisha was randomly screened for haemoglobin variants and G6PD deficiency and high frequencies of sickle cell haemoglobinopathy (0-22.4%) and G6PD deficiency (4.3 to 17.4%) were found with 12 individuals inheriting both these abnormalities[4.] Among the 14 primitive tribal populations from four different States showing a high frequency of sickle gene, the prevalence of G6PD deficiency varied from 0.7 to 15.6 per cent[5] The first description of sickle haemoglobin in India was by Lehman and Cutbush in 1952 in the tribal populations in the Nilgiri hills in south India[6]

MATERIALS AND METHODS:

The suspected blood samples were collected from Aditya Multicare Hospital, and Omni RK hospital, Visakhapatnam. The accepted laboratory practice for diagnosis of Sickle Cell Anemia is the preparation and microscopic examination of blood films. Sickling of the red blood cells, on a blood film, can be induced by the addition of sodium metabisulfite. Hemoglobin electrophoresis and hemoglobinocyanide method are also used in the determination of hemoglobin levels. Electrophoresis seperates normal and abnormal types of Hb. Citrate agar gel electrophoresis is preferred here (Ph8.4).Each Hb have different charges and different electrophoresis mobility. Different types of stationary phases are selected for the detection and separation of components of hemoglobin[8].It is more sensitive than cellulose acetate membrane electrophoresis(ph6.5) for detecting Hb S and Hb F. Estimation of hemoglobin using haemoglobinocyanide make the detection of amount of hemoglobin content present in the whole blood sample. This dilution technique is useful in detecting the anemic conditions and any other abnormal disorders of Hb which affects the amount of it. when the whole blood sample is taken in a test tube having haemoglobinocyanide, the RBC gets lysed immediately and Hb elutes out of the cell. This can be measured by using a colorimeter at 530nm. In

PCR, the isolated DNA from the test and control samples by using phenol/chloroform method. The DNA pellet is suspended in the TE Buffer. Specially designed primer sets for the confirmation of abnormal hemoglobin in the blood sample is selected. These primers are suspended in TE buffer. The dNTPs , 5x buffer, Taq polymerase and distilled water at appropriate volumes are taken and all the contents were mixed amd taken in PCR vials and allowed to undergo the amplification. After amplification they are placed on agarose gel to observe the bands. Determination of G6PD Activity

RESULT:

in RBCs involves the release of G6PD by a lysating agent present in the starter reagent. G6PD catalyses oxidation of glucose-6p with reduction of NADP to NADPH. The rate of its reduction is measured as an increase in absorbance which is directly proportional to G6PD activity in the sample**.**

Normal values: - 4.6 to 13.5U/g Hb

$$\Delta A/\text{Min} = \frac{A2-A1}{5}$$

CALCULATION :- G6PD ACTIVITY(U/ 10^{10} RBC) = $\Delta A \times \frac{4778}{\text{Hb g/dl}}$

Fig 1:- Microscopic examination of sickle cell anemia positive cases having sickle shape cells which differentiate between normal RBC, having bi concave shape.

Fig 2:- citrate agar gel electrophoresis shows seperation of components in Hb , detecting the normal and abnormal bands.

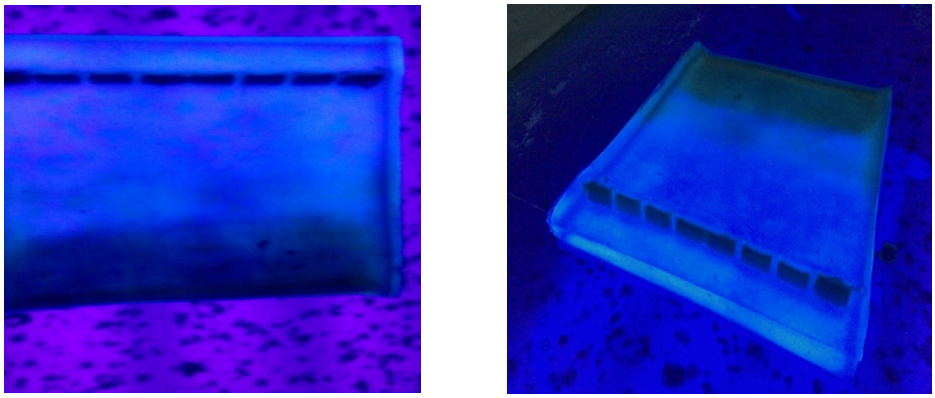


Fig 2.1:- After the electrophoretic run, the bands observed on the gel are as follows.

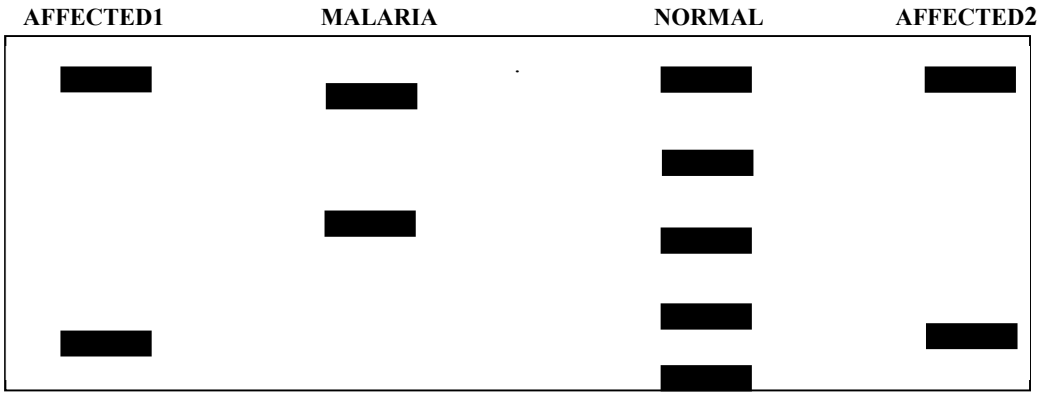
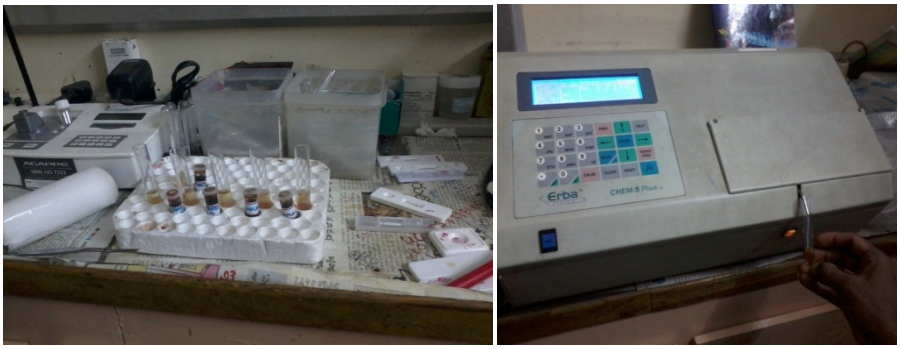


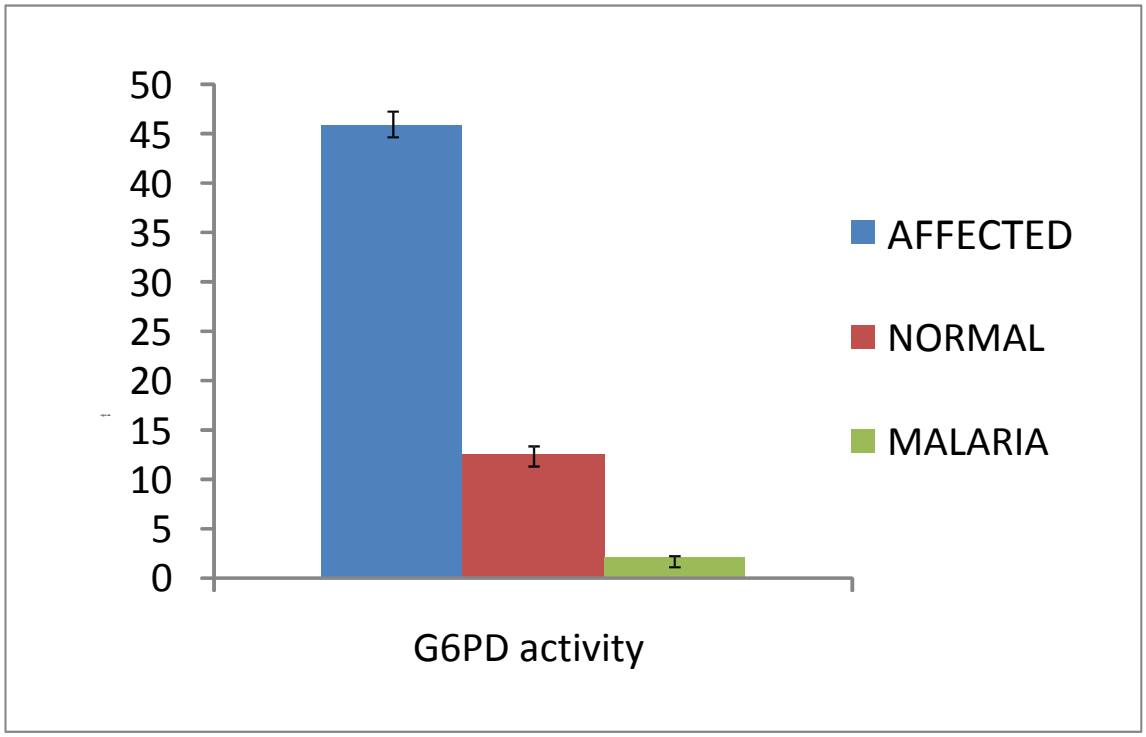
Figure 3:- Estimation of Hemoglobin by adding hemoglobinocyanide to the blood sample.

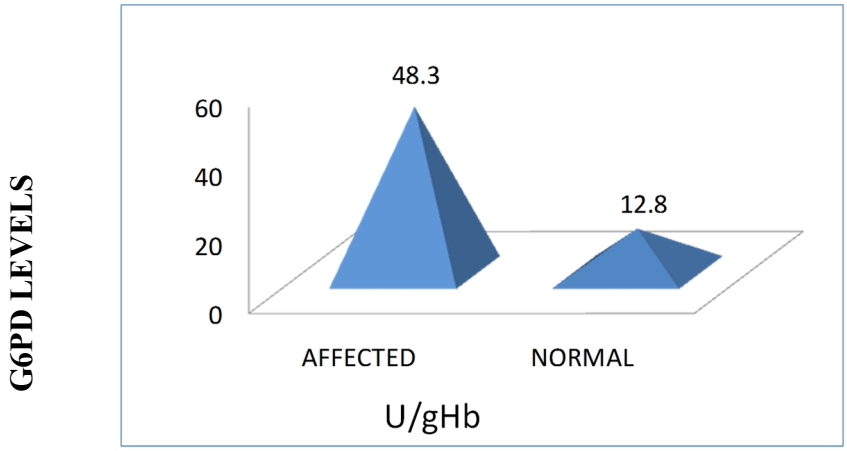


Age group	sickle cell patients (affected)	
(years)	number(n)	G6PD values (U/gHb)
10-May	3	22.6± 8.3
20-Oct	6	36.4± 6.5
20-30	7	46.4± 5.7
30-40	3	34.6±2.6
40-50	6	2.8 ± 0.03
50-60	5	1.8 ± 0.01
Age group	Normal samples	
(years)	number(n)	G6PD Values (U/gHb)
10-May	3	9.4±0.12
20-Oct	6	11.9±0.25
20-30	7	13.2±0.21
30-40	5	12.4±0.11
40-50	6	7.3±0.16
50-60	3	5.6±0.28

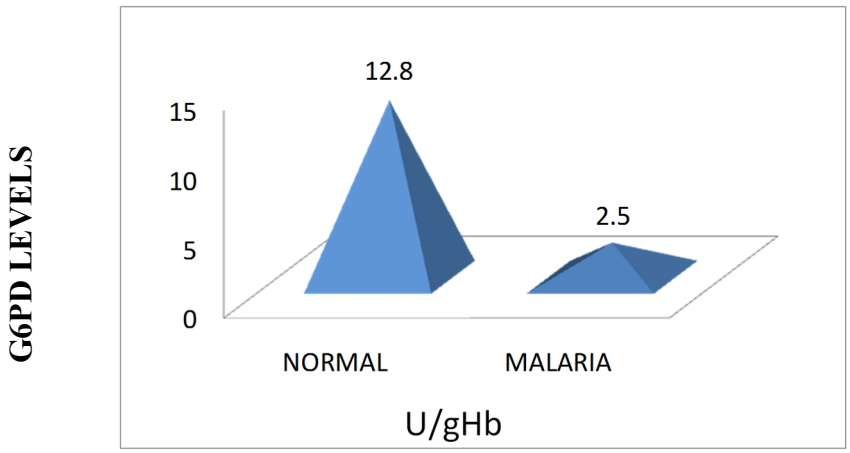
	SICKLE CELL		NORMAL		MALARIA	
AGE GROUP	ANEMIA					
	number(n)	G6PD values (U/gHb)	number(n)	G6PD values (U/gHb)	number(n)	G6PD Values (U/gHb)
10-May	3	22.6±8.3	3	9.4±0.12	3	1.5±0.01
20-Oct	4	36.4±6.5	6	11.9±0.25	6	2.0±0.03
20-30	7	46.4±5.7	7	13.2±0.21	7	1.9±0.04
30-40	3	34.6±2.6	3	12.4±0.11	3	1.2±0.03
40-50	6	2.8±0.03	6	7.3±0.16	6	2.8±0.08
50-65	5	1.8±0.01	5	5.6±0.28	6	3.2±0.01

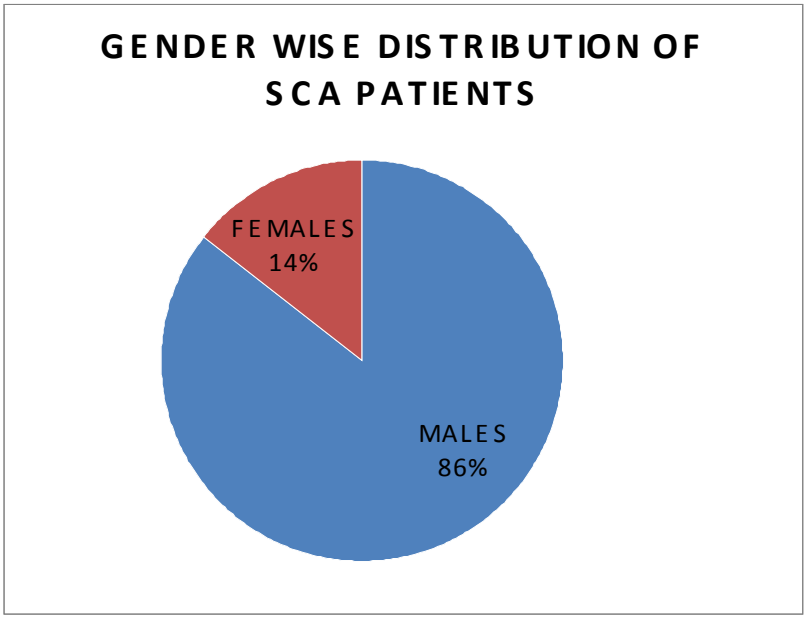
G6PD levels

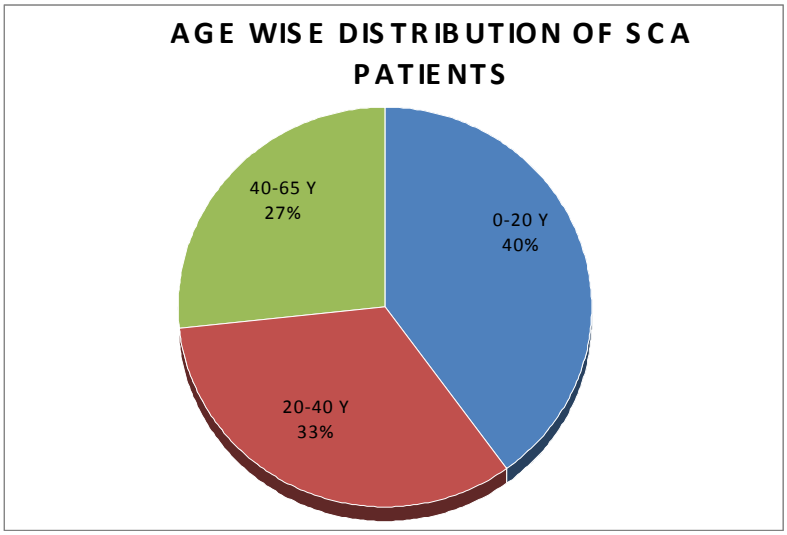




normal Vs malarial subjects







DISCUSSION:

Glucose is the main source of energy for the red cell, which is metabolized by two major routes; the glycolytic pathway and the hexose monophosphate (HMP) shunt. Glucose-6-phosphate-dehydrogenase (G6PD) is an X-linked enzyme that catalyses the first step in the HMP pathway of glucose metabolism and it produces NADPH, which is required for the maintenance of reduced glutathione (GSH). GSH is essential for protecting red cells from oxidative damage[7]. Hence, this enzyme is important in red cell metabolism and its deficiency renders the red cell extremely vulnerable to any kind of oxidative stress. Our study is based on detecting the abnormal Hb levels in the person, along with the detection of G6PD levels which is an important diagnostic method in detecting sickle cell anemia. Microscopy is a basic test identifies the presence of sickle cell trait, but it is unable to confirm the presence of disease.. It specifies which gene is responsible in causing SCA.Estimation of G6PD enzyme in RBC‘s is another important diagnostic method in diagnozing the abnormal RBC. The affected RBC are having elevated levels of G6PD compared to normal samples in reticulocyte stage where as deprived levels in mature RBC stage. The malarial samples however deficient of the enzyme as the parasite utilizes it for its metabolism. Hence the levels of the enzyme will decrease from the starting stage to the cell death. The results obtained are matched with previous studies confirms that

G6PD activity is another important method in diagnosing the disease[10].

CONCLUSION:

G6PD activity is a newly emerging method which also diagnoses abnormal hemoglobin and also detects the severity of disease. Further studies are needed to definitively establish the mechanisms by which G6PD deficiency confers an advantage against malaria in heterozygous individuals. Such studies could lead to the development of new treatments. G6PD deficiency has different effects against different subtypes of severe malaria, predisposing to severe malarial anaemia but protecting against cerebral malaria. Moreover, these subtypes vary with malaria transmission intensity and thus between populations and over time, meaning that the net evolutionary pressures for G6PD deficiency might be more complex than previously appreciated.

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