

# Fetal haemoglobin: A novel prognostic determinant in sickle cell anaemia

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**Abstract:** *Background-Acknowledgement of instrumental action of fetal hemoglobin(HbF) in improving the clinical condition of sickle cell disease (SCD) patients, began around 7 decades back, by Janet Watson who found that infants with SCD had lesser symptoms and their deoxygenated RBCs did not sickle or deform as aggressively as their mothers having sickle cell trait. She proposed high HbF levels in infant blood as compared to mothers for the observations of her study. Reduced incidence of crisis like vaso-occlusive, haemolytic, aplastic, megaloblastic, infectious and others have been found with higher HbF levels. With this backdrop the present study is being undertaken to know the reciprocity between HbF levels and the state of crisis in sickle cell. The study is expected to fill the missing link between “Why a few patients with HbS with haemoglobin pattern of SS suffer less from crisis than that of others with a similar haemoglobin pattern of SS”*

**Objectives:**

- *To know HbF levels in patients with SCD with Hb (SS) pattern*
  - *To draw the comparisons between HbF levels in SCD with Hb(SS) pattern in subjects 1. With sickle cell crisis and 2. Without sickle cell crisis*
  - *To find out cut-off level of Hb F at its protective effect against the crisis in SCD*
- Methodology**
- *Study design - Observational and analytical study*
  - *Sample size - Total of 30 Patients of sickle cell Disease (Hb SS)*
  - *Study duration - 1 year*

**Expected Results:** *Incidence of Crisis in HbSS patients are expected to have a inverse relationship with HbF levels. A cut-off level of HbF above which episodes of crisis are lower can be expected to be found. Conclusion: Conclusions will be drawn from results obtained and an attempt to establish a cut-off HbF value which renders a protective action against crisis in SCD will be made.*

**Keywords:** *HbSS, SCD, Foetal Haemoglobin, sickle cell disease, crisis.*

## **Introduction:**

Sickle cell anemia remains the most commonly encountered hemoglobinopathy and a major health burden to deal by healthcare providers.<sup>1,2</sup> It peculiarly different than other anemias as it in its natural course of disease is associated with situations of crisis.<sup>2,3</sup>

The common crisis encountered in clinical practice in sickle cell anemia includes vaso-occlusive, aplastic, haemolytic, megaloblastic, infectious and many others.<sup>4</sup> These crisis are often happening because of rapid polymerization of HbS in the RBCs of sickle cell anemia subjects, based on these several severity scores have been proposed.<sup>5,6</sup>

In recent years the publications in medical literature have proposed that fetal haemoglobin (HbF,  $\alpha_2\gamma_2$ ) have inhibiting effect over deoxygenation induced polymerisation of sickle Hb (HbS,  $\alpha_2\beta^S_2$ ). A few reports have stressed that the more are the RBCs with Hbf the lower is the crisis reported in the subjects with SCD. <sup>7,8,9,10,11</sup>

The persistence of HbF in RBCs modulates contact between RBCs with HbS and inhibits polymerisation in deoxygenated RBCS.<sup>6</sup> The fraction of RBCs dominated by HbF cells that contain around 10 picogram of HbF is abled for inhibition of polymerisation in toto, even at oxygen saturation levels of 40 to 70%. In other terms the higher HbF content of RBCs the longer the survival span. <sup>12,13,14,15,16,17</sup>

There are reports in literature that have inferred, that there is an inverse relationship for crisis in sickle cell anemia with increased HbF hemoglobin content. In other words these studies have concluded that higher the HbF content lower is the crisis in sickle cell anemia patients. <sup>7,8,9,10,11</sup>

### Knowledge Gap

With this backdrop the present study is being undertaken to know the reciprocation between HbF levels and the state of crisis in sickle cell anemia which has yet not been studied in a length in indian population of sickle cell anemia patients which requires to be bridged. The study is expected to fill the missing link between “Why a few patients with HbS with haemoglobin pattern of SS suffer less from crisis than that of others with a similar haemoglobin pattern of SS” and establish a cut-off level of HbF that renders protective action against crisis

### HYPOTHESIS

The hypothesis constructed is HbF has a protective and inhibitory effect over the polymerisation of HbS in event of sickle cell anaemia crisis.

### Research Questions

Do levels of HbF predicts the crisis in patients of SCD (HbSS pattern)

### Aim

To study HbF presence and its levels in the patients of SCD(HbSS pattern)

### Objectives

1. To detect HbF in pateints with SCD.
2. To compare HbF levels in patients with SCD with and without crisis.
3. To establish HbF levels in SS pattern SCD patients as determinant of crisis.

### Material and methods-

Place of study- Division of Hematology in Department of Pathology JNMC Sawangi (Meghe) Wardha.

Duration of study- 1 year

Sample Size- 30 patients of sickle cell anaemia (Hb SS) was calculated using the Krejcie and Morgan Methodology.

Sample collection and storage : Sample will be collected in EDTA bulbs at central laboratory OPD or wards of AVBRH Sawangi, Meghe, Wardha. Sample will be processed within four

hours post collection, but if need due to any delay in processing samples will be stored in laboratory fridge at 2- 4 Degree Celsius.

Study design- Observational and analytical study.

Patient characteristic

Inclusion criteria-

1. Sickle cell anaemia patients without crisis having SS pattern on Hb electrophoresis or HPLC.
2. Sickle cell anaemia with crisis (vaso-occlusive, aplastic, haemolytic, megaloblastic, infectious and many others) having SS pattern on Hb electrophoresis or HPLC.

Exclusion criteria-

1. Sickle cell anaemia patients with Hb AS pattern on Hb electrophoresis or HPLC.
2. sickle cell anaemia with other combined hemoglobinopathies.

Consent:-

The consent will be obtain of all the patients as per the AVBRH policy.

Technique:-

- A. Haemoglobin electrophoresis by cellulose nitrate and band elutions for quantitative estimation for HbF.
- B. Alkali denaturation test and quantization of HbF.
- C. HPLC

Materials:-

- EDTA Bulbs
- Centrifuge
- Electrophoresis apparatus (Electrophoresis Tank, Applicator)
- Cellulose nitrate/acetate paper.
- Power source (350V at 50mA)
- Tris phosphate buffer
- 1% NAOH

Comparison:-

1. Thirty patients of sickle cell anemia will be divided in two groups  
Group A- SCD cases without crisis  
Group B- SCD cases with crisis
2. The HbF levels between these two groups will be estimated and compared

Statistical Analysis: -

The levels of HbF will be compared by statistical means as follows (Mann-Whitney U Test)

1. SCD cases without crisis, and sickle cell anaemia with crisis.

2. SCD cases for its type of crisis.

Implication:

1. The HbF levels are implied as prognostic and predictive indicator for sickle cell anaemia crisis.
2. HbF level detection to be included in the workup of sickle anaemia patients for treatment and management.

Observation and Discussion: It will be performed in suitability with objectives and methods.

Expected Results:

Incidence of Crisis in HbSS patients are expected to have a inverse relationship with HbF levels. A cut-off level of HbF above which episodes of crisis are lower can be expected to be found.

Discussion:

Oluwagbemiga o et al did a study in 2017 on “fetal hemoglobin and disease severity in Nigerian children with Sickle Cell anemia” in their cross-sectional study they determined the reciprocation between HbF levels and severity of SCD among Nigerian children in the age group of 1 to 15 years with SCD. The SCD severity was evaluated on the basis of frequency of episodes of pain, blood transfusions required and hospital admission in the 12month period of study, past cumulative frequency of complications due to SCD, degree of enlargement of spleen and liver, hematocrit and WBC count.<sup>17,18</sup>

In their study they found the average HbF level in their subjects was  $9.9 \pm 6\%$ . Females had significantly higher mean HbF levels than males,  $12.2 \pm 5.8\%$  vs.  $8.0 \pm 5.6\%$  ( $p < 0.001$ ). No case had severe disease. However, the 32 cases with moderate disease had significantly low mean HbF levels as compared to the 73 cases with mild disease ( $7.7 \pm 5.6\%$  vs  $10.8 \pm 6\%$  respectively). Mean Haemoglobin F level was also significantly lower in cases having history of acute chest syndrome and stroke than to those without these complications.<sup>17</sup>

They concluded that cases with SCD who had a moderate severity of SCD and those with a history of life-threatening crisis conditions such as acute chest syndrome and stroke had significantly lower HbF levels. Therefore, it was recommended by them that early quantification of foetal haemoglobin and HbF inducement should be made available to reduce the severity, morbidity and mortality among cases of SCD.<sup>17</sup>

Related studies were reported. Goyal et al reported estimation of plasma haemoglobin by a modified kinetic method using o-tolidine<sup>19</sup>. Pareek et al reported on correlation between nephropathy and ophthalmic complications in cases of sickle cell anemia<sup>20</sup>. Verghese et al assessed clinical profile of patients of sickle cell crisis in a rural tertiary care hospital<sup>21</sup>. Agrawal et al reported about Sickle Cell Crisis Leading to Extensive Necrosis<sup>22</sup>. Related studies were reported by Bhagat et al<sup>23</sup>, Mzezewa, S et al<sup>24</sup> and Khatib et al<sup>25</sup>.

**Conclusion:** Conclusions will be drawn from results obtained and an attempt to establish a cut off HbF value which renders a protective action against crisis in SCD will be made.

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