SICKLE CELL DISEASE: REAPPRAISAL OF THE ROLE OF FOETAL HAEMOGLOBIN LEVELS IN THE FREQUENCY OF VASO-OCCLUSIVE CRISIS

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SUMMARY

Background: Foetal haemoglobin has been implicated in the modulation of sickle cell crisis. Its level is generally inversely proportional to the severity of sickle cell disease (SCD) for a given sickle cell phenotypes. The main aim of therapy for vaso-occlusive crisis (VOC), which is the hallmark of SCD, is to reduce the chances of sickling through the prevention of polymerization of HbS. One way of preventing this polymerization is by increasing foetal haemoglobin levels.

Objectives: To determine the relationship between HbF levels and the frequency of crisis in SCD patients in Ghana.

Method: A longitudinal retrospective survey covering a period of 30 months was carried out on adult SCD patients at the Sickle Cell Clinic of the Korle-Bu Teaching Hospital.

Results: Eighty-three adults aged 15 to 65 years made up of 40 males and 43 femalea were studied. Analysis of variance (ANOVA) gave significant results in Hb and HbF levels. Higher HbF levels were positively related to less frequent crisis and were significantly high in SCD patients than in controls. HbF effects on the clinical manifestations on SCD were variable.

Conclusion: Threshold values of HbF play a role in reducing the frequency of vaso-occlusive crisis in SCD patients and this finding contributes to the body of available literature on SCD severity. However our work does not give the apparent threshold level of helpful HBF Level in SCD.

Keywords: Haemoglobin F, Frequency of crisis, sickle cell disease.

INTRODUCTION

Sickle cell disease (SCD) is a major genetic disorder in tropical Africa.[1]In Ghana the prevalence rate is 1.9% of all births per year[2] and is responsible for significantly high rate of morbidity and mortality in Ghana. SCD placed 37th and 36th positions in the years 2002 and 2003 respectively on the out-patient morbidity reports compiled by the Ghana Health Service. Even though clinical manifestation of SCD displays a wide array of symptoms, recurrent attacks of VOC are responsible for most of the morbidity and mortality in SCD.[2, 3]

Pathophysiologically, oxygenated Haemoglobin A (HbA) and Haemoglobin S (HbS) has the same solubility, but on deoxygenation, HbS is about 100 times less soluble.[3]The deoxygenated Haemoglobin S polymerizes by sticking to each other to form long strands, which stretches the membrane of the red blood cell.[4,5, 6] This defect causes polymerization of haemoglobin molecules and erythrocyte sickling under hypoxic conditions[7, 8, 2] leading to VOC. VOC is caused by sickled red blood cells which obstruct capillaries and restrict blood flow to an organ, resulting in ischemia, pain, and organ damage[9, 10] and it is responsible for the high rate of morbidity[3] and mortality[2] in SCD.

The disease severity depends primarily on the genotype which is ranked as HbSS > HbS/β°-thalassemia > HbSC > HbS/β^+-thalassemia.[11] The severity of the disease is also related to β-globin haplotypes, possibly due to variations in hemoglobin level and fetal hemoglobin concentrations. Nevertheless, haplotypes of the β-globin gene cluster and α-globin complement affecting the expression of factors such as HbF, modulate the disease severity.[12] Five haplotypes of the HbS allele have been identified from various populations based on two polymorphic clusters.[12]

These clusters are thought to correlate with hematological markers and HbF gene expression leading to a decreasing disease severity as Bantu > Cameroon > Benin > Arab Indian > Senegal[13] and are geographically restricted.

However, work done by Inati et al., and a recent work[14] indicated that there are still atypical haplotypes that do not conform to literature.[15] For instance, in one report from Lebanon, high HbF concentration was associated with increased disease severity[16], a finding which warrants further investigation.

Some genetic and environmental factors have been identified that ameliorate the severity of the SCD condition. The most important of these is a high level of hemoglobin F (HbF) in the erythrocytes.[17] The first insight into the role of foetal hemoglobin in the clinical manifestations of SCD was made by a pediatrician, Janet Watson.[18] Watson and her colleagues at a New York hospital noted that babies with SCD rarely had manifestations of the condition in their first year of life.[18] They proposed that the high level of HbF in the red cells, which persists during the first year of life, somehow protected the infant. Foetal Hb levels decline to their low levels of less than 2% six months after birth.[19] The childhood manifestations of SCD are seen thereafter.

Clinical observations have also confirmed that increased foetal haemoglobin concentrations have beneficial effects in SCD.[20, 21, 22] Patients with SCD who have elevated HbF levels have fewer painful crises and improved survival[20]as shown by studies that fetal haemoglobin, which lacks β -globin chains, inhibits sickling in vitro by interfering with the polymerization of haemoglobin S[23, 24]. Since polymerization of deoxy-HbS is the signal event in the pathogenesis of SCD[4], HbF could effectively prevent disease manifestation.

Patients with sickle cell disease who also have hereditary persistence of foetal haemoglobin (HPFH) often have few if any symptoms[19]. In these individuals, HbF usually comprises greater than 20% of the haemoglobin in the erythrocytes. It has been documented that increased HbF levels ameliorate the severity and frequency of painful crisis in SCD patients.[20, 21, 22] Studies within populations of African origin have also shown that high HbF levels are associated with milder disease.[26, 27, 28]

However, the role of HbF on the clinical sub phenotype is variable and inconsistent such that even levels nearing20% may be found in patients with severe disease. The threshold for significant reduction in acute episodes of pain, chest syndromes, and priapism is 20%, and for organ damage, 10%.

A retrospective study in Jamaica did not find significant difference in painful episodes or acute chest syndrome between different levels of HbF level,[29] whilst no protective effect of HbF was found in studies by Baum.[30]However, a clear protective effect was found in the Co-operative Study in the USA[17, 21, 31]also found no linear relationship between HbF and clinical severity, but proposed that there were `threshold levels' above which HbF was an ameliorating factor (10% for stroke, 20% for recurrent events such as painful crises).

Several cytotoxic agents such as hydroxyurea[25, 32] and 5-azacytidine[33,34] enhance the levels of foetal hemoglobin developing erythroid cells. Hydroxyurea has been administered to patients with sickle cell disease in an effort to enhance foetal hemoglobin production.[35] Hydroxyurea induces foetal haemoglobin production[35-38] increases the red cell mean corpuscular volume, and reduces the number of dense cells and irreversibly sickled cells in the circulation,[24] thereby reducing morbidity and mortality in SCD patients.[38]Treatment with hydroxyurea caused a 44%reduction in the median annual rate of painful crises in patients with SCD.[23]

The possible benefit of HbF in reducing the frequency of crisis in SCD in Ghana is not clear. This study will give a better understanding of the relationship between HbF levels and the frequency of crisis in SCD.

MATERIALS AND METHODS

The study was limited to patients with the HbSS and HbSC phenotypes. Voluntary blood donors at the National blood bank, Korle-bu Teaching Hospital were recruited for the study. Blood samples were screened for Sickle cell haemoglobin and those with sickle cell trait (HbAS as well as HbAC) were excluded. Five ml of venous blood was collected from the subjects into EDTA tubes for sickling test, Haemoglobin Electrophoresis, HbF and haemoglobin concentration determinations.

Sickling test for controls (screening) was by sodium metabisulfitemethod.[40] Measurement of HbF levels were by alkaline denaturation method as described by Singer and others.[41] VOC was clinically defined as pains in the bones, muscles and joints not attributable to any other cause and requiring parenteral analgesia and admitted in the Centre for more than an hour. The Frequencies of VOC was rerecorded by the number of times patients were hospitalized during the period of the study by a questionnaire.

All subjects gave their informed consent. Ethical clearance for this study was given by the Ethical and protocol review Committee of the University of Ghana Medical School.

RESULTS

Paired and unpaired student t-test was used to compare differences between and within sex-matched means in the groups, but these were not statistically significant. However, analysis of variance (ANOVA) gave significant results in Hb and HbF levels (Table 1).

Table 1 Haemorheological parameters in the studied groups

Males	SC_{20}	SS_{16}	AA_{83}	p-values
Age	29.6±13.1*	25.3±11.5	30.0±7.0	0.159
Hb	11.1±3.8	7.9±2.6	14.0±1.7	0.000
HbF	8.2±2.8	8.4±2.9	1.1±0.5	0.000
Females	SC_{26}	SS_{18}	AA_{19}	
Age	23.1±10.9	25.1±11.4	28.8±7.2	0.187
Hb	11.2±2.3	8.0±2.8	14.1±2.1	0.000
HbF	8.0±4.6	8.3±3.0	1.1±0.5	0.000

*Mean standard deviation; Sickle cell patients appear younger than controls but this was not statistically significant after analysis of variance was done; p-values less than 0.05 were said to be significant.

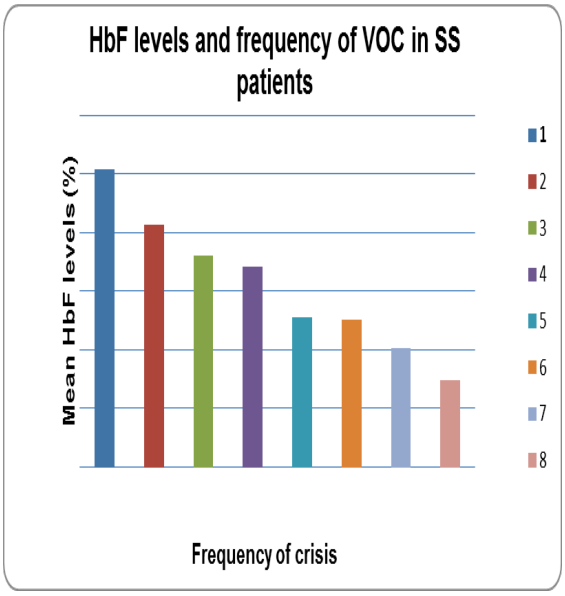

Figure 1 Hb F levels and frequency of vaso=occlusive crises in sickle cell Hb SS disease patients.

HbF effects on the clinical manifestations on SCD were slightly variable (Figures1 and 2).This conforms to previous studies that stated `threshold levels' above which HbF could be an ameliorating factor.[21, 29-31]

The higher the HbF level in an individual, the lower the frequency of crisis one experiences.

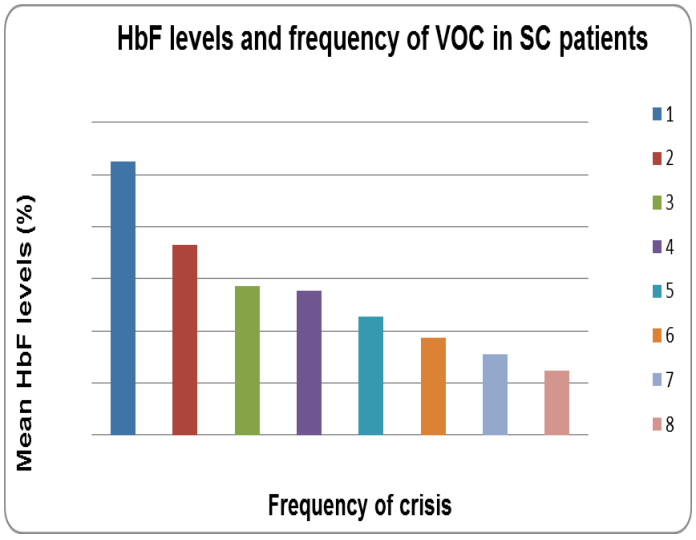

Figure 2 Hb F levels and frequency of vaso=occlusive crises in sickle cell Hb SC disease patients.

HbF appears to benefit some complications of disease more than others (Figures 1 and 2). This might be related to the premature destruction of erythrocytes that do not contain HbF, even though the total HbF concentration was high.

DISCUSSION

In this study, HbF levels were significantly higher in SCD patients than in controls. This was consistent with the finding of Konotey-Ahulu.[1] The high levels of HbF in SCD patients may suggest that increased foetal haemoglobin concentrations may have beneficial effects in SCD[22,24]This is because increased cellular levels of HbF reduces the tendency of deoxygenated HbS to polymerize since HbF interferes with the intracellular polymerization of HbS[42].Therefore, HbF confers a survival advantage to erythrocytes in proportion to the amount of HbF present[43] and has beneficial effects on patients with SCD.

HbF effects on the clinical manifestations on SCD were slightly variable. This conformed to previous studies that stated `threshold levels' above which HbF could be an ameliorating factor.[21, 25-26, 29-31]The particular threshold is still a subject of concern.

Furthermore, haplotypes of the β-globin gene cluster and α-globin complement affecting the expression of factors such as HbF, also modulate SCD severity. Haplotypes of the HbS allele have been identified from various populations of distinct polymorphic clusters.

These clusters are thought to correlate with hematological markers and HbF gene expression which impact on disease severity. To shed more light on these, more sensitive studies like high performance liquid chromatography for HbF determination and targeted genomic analysis are needed.

A limitation of our study was the small sample size that was comparable to literature cited. Nonetheless, these findings add to the small body of literature concerning SCD severity.

CONCLUSION

Threshold values of HbF play a role in reducing the frequency of vaso-occlusive crisis in SCD patients and this finding contributes to the body of available literature on SCD severity. However our work does not give the apparent threshold level of helpful HbF Level in SCD.

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