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Research Article

Markers of Disease Severity Amongst Homozygous Sickle Cell Anaemia Attending Outpatient At Bowen University Teaching Hospital Ogbomoso, Nigeria.

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Abstract

Introduction: Genetic, cellular and molecular modifiers are responsible for the notoriously variable sickle cell phenotype. Haemoglobin F is a principal modulator of the SCD phenotype. Haemoglobin F inhibits the polymerization of Haemoglobin S and ameliorates the secondary effects of sickling. We measured the Haemoglobin F(HbF) in our population and compared it with other markers associated with clinical severity and clinical status of the patients.

Methods: we randomly selected 40 Hemoglobin S(HbS) patients who have never taken hydroxyurea. We measured hemoglobin F levels, packed cell volume (PCV) and reticulocyte count to serve as markers for hemolysis, neutrophils, platelets and MCHC to serve as markers for clinical severity. We searched for a relationship between these laboratory features and frequency of vaso-occlusive crises, transfusion history and number of hospital visits per year. They were compared with twenty healthy Hemoglobin A (HbA) controls.

Results: Packed cell volume was significantly lower and the reticulocyte was significantly higher in the hemoglobin S compared with controls. The platelet count of the sickle cell anaemia patients was more than twice the number of the controls. The mean hemoglobin F level was 7.1± 3.5 %. The hemoglobin F was negatively correlated with the platelet count but positively correlated with the total white cell count and haematocrit but there was no significant correlation between hemoglobin F and clinical features

Conclusion: The fetal hemoglobin level may not be the only modifier responsible for phenotype in population of hemoglobin S

Introduction

Sickle cell disease (SCD) is a single gene disorder of red blood cell which presents with chronic hemolysis, vaso-occlusion and end organ damage. (1) This disease predisposes the patients to life-threatening complications, and extensive organ damage resulting in increased morbidity and reduced quality of life. (2) Worldwide, Sub-Saharan Africa has the highest prevalence of sickle cell anemia. In Nigeria, the prevalence of the sickle cell trait remains steady at 20 to 30%. (3) The homozygous sickle cell anemia affects about 2 to 3% of the Nigerian population. The most recent estimates in Benin City, Nigeria reconfirmed a Sickle cell anaemia prevalence of 2.39% and prevalence of the sickle cell trait of about 23%. (4)

The basis of the phenotype of sickle cell anemia is intracellular polymerization of Hemoglobin S upon deoxygenation. This polymerization makes the cells rigid, damaging cell membrane. When the damage to the cell is no longer reversible, the cell assumes a sickle shape. Microvascular occlusions and endothelial hyperactivity results

from this rigid sickle shape and predisposes the red cell to destruction by macrophages and monocytes in the reticuloendothelial system, culminating in a hemolytic anaemia and vasculopathy.(5) Factors interfering with S polymerization prevents initiation of this cascade. A handful of cellular and molecular factors reduce the polymerization of the Hemoglobin S. One of the most effective is the presence of other hemoglobin within the cell. Hemoglobin F and A_2 have been discovered to limit the Hemoglobin S polymerization to the greatest extent. From a clinical viewpoint, the effect of hemoglobin F on the clinical severity of sickle cell anaemia has been investigated and conclusions have been drawn. Higher hemoglobin F levels is strongly associated with fewer leg ulcers, fewer episodes of vaso-occlusive crises and longevity. Certain conditions such as compound heterozygotes for hemoglobin S and hereditary persistence of hemoglobin F have hemoglobin F concentrations of about 20% in each sickle erythrocyte. These populations are asymptomatic with only minor laboratory findings. (6) Hemoglobin A_2 is another hemoglobin that inhibits polymerization of S; elevated levels are seen in Sβ thalassemia. Sβ thalassemia have fewer dense and irreversibly sickled, increased erythrocyte lifespan compared with homozygous SS (7). Concerning cellular modifiers, leukocytosis is a risk factor for early death in adults and children. Leukocytosis, in the absence of infection, is common in Sickle cell anaemia and predicts the likelihood of stroke, acute chest syndrome, and an overall increase in mortality. Neutrophils and monocytes are activated in sickle cell anaemia. These activated leukocytes promote vascular inflammation and vessel damage. When these activated leukocytes are reduced, the frequency of sickling is reduced. (8) dehydration of red cells encourages polymerization of Hemoglobin S. A higher MCHC indicative of cellular dehydration has been positively correlated with the percentage of dense irreversibly sickled cells. We aimed to record the status of the pediatric sickle cell anaemia population in our environment and correlate clinical and laboratory features with disease severity.

Materials and Methods

The Bowen university Teaching Hospital Human Research Ethical Review Board approved this study. Informed consent was obtained from each parent or guardian of the subjects in addition assent was obtained from subjects older than 10 years of age. The patients were recruited using the simple random technique of sampling. Patients included in the study were those in steady state, gave consent and was diagnosed as homozygous SCD (Haemoglobin SS) which was confirmed by alkaline haemoglobin electrophoresis. An interviewer-administered questionnaire was used to obtain biographic and clinical information from the patients, some clinical information was extracted from the clinical records of the patient. The subjects were between the ages of 3-17years. Twenty apparently healthy children between the ages of 5-17years and who were Hemoglobin AA served as controls. Informed consent was obtained from the parents and for children older than 10years, assent was obtained in addition.

Five millilitres of venous blood were collected from the ante-cubital vein after cleaning with methylated spirit. The samples were separated into two different sample bottles. Both bottles one and two contained pottasium ethylene diaminetetra acetate (K EDTA) . Three millilitres of blood was put in sample bottle 1 and two millilitres was put in sample bottle 2 . Full blood count was run with bottle 1 within 30 minutes of sample collection using the Cell-Dyne 1200 haematology analyser. After which, Reticulocyte count was run with the blood from sample bottle 1. Sample bottle 2 was used to run Haemoglobin F using the high-performance liquid chromatographic method using Dionex UltiMate® 300 Rapid Separation LC System.

Statistical analyses were conducted using the IBM Statistical Package for Social Sciences (SPSS) ^{Tm} version 23.0 for windows. Means of normally distributed data were obtained. student T test was used to investigate the difference between means. Pearson's Correlates were used to investigate relationships between continuous variables. *P* values <0·05 were considered significant.

Results

A total of 40 patients were recruited into the study. Patient age ranged from 3 to 17 years (median 8.2 years) at the time of recruitment. Forty percent of the patients (40%) were males. The clinical characteristics of the patients with sickle cell anaemia are shown on table 1. Ten percent of the

patients presented with a diagnosed infection in the previous year, while only five percent were hospitalized within the past year. Table 2 compared the blood indices for sickle cell anaemia with controls. The total white cell count and platelet count are significantly higher in the sickle cell group while the haematocrit and mean cell volume is significantly lower in the sickle cell group. The platelet count of the sickle cell anaemia patients was more than twice the number of the controls (444.6 X 10^9 /L vs 208 X 10^9/L). The Reticulocyte count was approximately six times higher in the study group compared with the control. The mean hemoglobin F level was 7.1± 3.5 % .(Hb F for Hb A 0.5-1.5%) The hemoglobin F was negatively correlated with the platelet count but positively correlated with the total white cell count and haematocrit as noted on Table 3

Table 1: The clinical characteristics of sickle cell anaemia patients.

Clinical characteristics	Response	Number	Percentage%
Fever in the last year	Yes	4	10
	No	36	90
Diagnosed Infection in the last year	Yes	4	10
	No	36	90
Number of bone pain crises in the last year	None	26	65
	1	10	25
	2	2	5
	≥ 3	2	5
Transfusion history	Yes	3	2.5
	No	37	92.5
History of hospitalization in the past one year	Yes	5	12.5
	No	35	87.5

Table 2: Blood indices of sickle cell anaemia patients

Full Blood count	Study n=40 mean(sd)	Control n=20 mean(sd)	T value	P value
Haematocrit (%)	24.3(3.9)	32.0(10.6)	-3.2	0.003
White blood cell count (×10^9/L)	18.6(8.6)	5.9(2.4)	6.3	0.000
Neutrophil count (×10^9/L)	10.1(4.0)	2.6(1.0)	8.04	0.000
Platelet count (×10^9/L)	444.6(201.2)	208.3(63.5)	5.0	0.007
Mean Corpuscular Volume (fL)	76.9(10.2)	84.2(5.6)	-2.9	0.000
Mean corpuscular Haemoglobin Concentration (g/L)	26.3(3.2)	31.7(1.5)	-6.8	0.004
Reticulocyte count %	12.02 ± 10.69	2.80 ± 2.04	2.054	0.057

Table 3: Relationship between Haemoglobin F and blood indices in HbS

Parameter	R	P value
White blood cell count (x 10^9/L)	0.19	0.70
Neutrophil count (x 10^9/L)	-0.14	0.60
Platelet count (x 10^9/L)	-0.10	0.81
Haematocrit %	0.32	0.17
Number of painful crises/year	0.32	0.17

R: Pearson correlation co-efficient

Discussion

This small hospital based study was carried out at Bowen University Teaching hospital Ogbomoso to characterize the sickle cell anaemic patients that live in Ogbomoso. Parameters that mark disease severity were measured and compared with controls. A similar study in Jamaica to characterize the hematology profile of sickle cell anaemia patients attending the hematology clinic had similar findings. The white cell count, neutrophil count and platelets of the subjects were significantly higher than controls and haematocrit

and mean cell volume were lower than in controls. The packed cell volume and mean corpuscular volume increased with increasing Hemoglobin F, but higher fetal hemoglobin had no effect on the incidence of painful episodes. (9) The observed haematological profile of the sickle cell anaemia in this study found a higher total white cell count, neutrophil count, and platelet count in sickle cell anaemia patients when compared with their hemoglobin A counterpart. The haematocrit and the mean corpuscular volume were however lower in the sickle cell anaemia subjects when compared with HbAA controls. While the findings concerning the haematocrit was expected, low mean corpuscular volume as a feature of sickle cell anaemia has only recently been accepted by clinicians in developing countries where both sickle cell anaemia and microcytosis are prevalent. (10) There are many mechanisms proposed for the presence of leukocytosis (without infection) in sickle cell anaemia, possibilities include chronic hemolysis, chronic pain and the anxiety that comes with chronic pain syndromes. (11) Leukocytosis is associated with an increase in morbidity amongst the sickle cell anaemia population. (12, 13). The lower haematocrit values in sickle cell anaemia is due to the chronic hemolytic state of the condition together with what has been described as a blunted response to erythropoietin. The effect is a smaller and slower rise of erythropoietin compared with the level of anaemia. (14) There is a significant rise in platelet count in sickle cell anaemia. This has been interpreted as due to lack of physiologic sequestration of platelets in sickle cell anaemia. (15) The mean corpuscular volume in our study mimicked iron deficiency amongst patients with sickle cell anaemia. There is variable data on iron status in sickle cell anaemia. Sani et al measured the iron status of patients in Ilorin, Nigeria and found that the subjects were iron replete (16) while Patel et al, in India discovered that iron deficiency may be found in children with sickle cell anaemia who have not had previous blood transfusions. He related the iron deficiency to high iron deficiency and malaria infection in the community.(17) Our study showed a microcytic anaemia in the study group despite a transfusion history in 2.5% and in consonance with findings by Patel et al. Reticulocyte count was significantly higher in the Hb S group confirming the chronic haemolytic state.

From this study we discovered that our population of hemoglobin S , have a fairly good clinical state. Only 2.5% of the sickle cell anemia patients had been transfused, only 10% presented with infections in the preceding year and 12.5% was hospitalized in past year. While these clinical features suggest a general good clinical state, the hematologic indices are not representative. We correlated the hemoglobin F levels with aspects of clinical state specifically bone pain crises to see if it could account for the clinical status of our study population. Patel et al found that marked variability in the hemoglobin F levels amongst hemoglobin S haplotypes is a feature which he concluded was responsible for the marked phenotypic variation. In his study group the levels ranged from <1% to 50%. When hemoglobin F levels are lower, more irreversibly sickled cell are found. (18) This suggests that the sickle cell population who do not have a particularly high concentration of Hemoglobin F should have a worse clinical course of the condition. In a study by Shaikho et al, hemoglobin F levels in Africans were 10%.(19) Our study found similar levels. We did not find any significant association between hemoglobin F and hematological indices or number of vaso-occlusive episodes in this population. We then concluded that the sickle cell anemia patients in our population have hemoglobin levels expected for African but appear to have a good clinical course.

Conclusion.

The HbS population studied had hematologic indices found typically in Sickle cell groups and a modest HbF levels but they appear to have a good clinical course. There may be other genetic or environmental modifiers responsible for the good clinical course.

References

1. Ballas SK, Kesen MR, Goldberg MF, et al. Beyond the definitions of the phenotypic complications of sickle cell disease: an update on management. Scientific World Journal. 2012;2012 :949535.
2. Pereira SA, Brener S, Cardoso CS, Proietti AB. Sickle Cell Disease: quality of life in patients with hemoglobin SS and SC disorders. Rev Bras Hematol Hemoter. 2013;35(5):325-31.

3. Akinyanju OO. A profile of sickle cell disease in Nigeria. Ann NY Acad Sci 1989;565:126-136
4. Nwogoh B, Adewoyin AS, Iheanacho OE, Bazuaye GN. Prevalence of haemoglobin variants in Benin City, Nigeria. Ann. Biomed. Sci. June. 2012;11(2):60–64
5. Pawliuk R, Westerman K A, Fabry ME, Paven E, Tighe R, Bouhassia EE et al. Corection f sickle cell disease I transgenic mouse models by gene therapy. Science. 2001, 294, 2368-2371
6. Akinsheye, I., Alsultan, A., Solovieff, N., Ngo, D., Baldwin, C. T., Sebastiani, P., Chui, D. H., & Steinberg, M. H. (2011). Fetal hemoglobin in sickle cell anemia.*Blood*, 118(1), 19-27
7. Steinberg MH, Sebastiani P. Genetic modifiers of sickle cell disease. Am J Hematol. 2012;87:824–826
8. Wun T. the role of inflammation and leukocytosis in the pathogenesis of sickle cell diseas ; hemoglobinopathy. Ematology.2001;5 50:403-412
9. Donaldson A, Thomas P, Serjeant BE, Serjeant GR. Foetal haemoglobin in homozygous sickle cell disease: a study of patients with low HBF levels. Clin Lab Haematol. 2001;23:285–9
10. Akinbami A, Dosunmu A, Adediran A, Oshinaike O, Adebola P, Arogundade O. Haematological values in homozygous sickle cell disease in steady state and haemoglobin phenotypes AA controls in Lagos, Nigeria. *BMC Res Notes*. 2012;5:396. Published 2012 Aug 1. doi:10.1186/1756-0500-5-396
11. Milhorat AT. Leucocytosis during various emotional states. Arch Neurol Psych. 1942;47:779.
12. Anyaegbu CC, Okpala IE, Aken'ova AY, Salimonu LS. Peripheral blood neutrophil count and candidacidal activity correlate with the clinical severity of sickle cell anaemia. Eur J Haematol. 1998;60:267–8.
13. Platt OS, Brambilla DJ, Rosse WF. et al.Mortality in sickle cell disease-life expectancy and risk factors for early death. N Engl J Med. 1994;330:1639–1643. doi: 10.1056/NEJM199406093302303
14. Sherwood JB, Goldwesser E, Chilcoat R. et al.Sickle cell anaemia patients have low erythropoietin levels for their degree of anaemia. Blood. 1987;67:46–49.
15. Freedman, ML, and S Karpatkin. "Elevated platelet count and megathrombocyte number in sickle cell anemia." *Blood* 46.4 (1975): 579-582.Web. 25 Mar. 2019
16. Musa A. Sani, James O. Adewuyi, Abiola S. Babatunde, Hannah O. Olawumi, and Rasaki O. Shittu, "The Iron Status of Sickle Cell Anaemia Patients in Ilorin, North Central Nigeria," Advances in Hematology, vol. 2015, Article ID 386451, 5 pages, 2015. https://doi.org/10.1155/2015/386451
17. Patel C, Jha BM, Jana S, Singh A, Shah H. Iron status in sickle cell disorders. Int J Med Sci Public Health 2016;5: 1759-176
18. Steinberg M, Chiu D H, Dover P S, Alsultan A. Fetal haemoglobin I sickle cell anaemia: glass half full? Blood, 2014, 123 (4): 481-485
19. Shaikho EM, Farrell JJ, Alsultan A, Sebastiani P, Steinberg MH. Genetic determinants of HbF in Saudi Arabian and African Benin haplotype sickle cell anemia. *Am J Hematol*. 2017;92(9):E555–E557. doi:10.1002/ajh.24822