

Determinants of Erythropoietin Level in Steady-state Homozygous Sickle cell Anemia

Dr. Lamis Kaddam^{1*}, Professor Imad Fdle Almula,², Dr Nada Hassan Eltayeb¹, Professor Amal M.Saeed⁴

1. Department of physiology, Faculty of Medicine Alneelain University Khartoum, Sudan.
2. Faculty of Medicine Alneelain University Khartoum, Sudan, Assafa Academy
3. Department of Physiology , Faculty of Medicine University of Khartoum –Khartoum-Sudan

Corresponding author: Lamis Kaddam email: lamiskaddam@hotmail.com

P.O. Box: 11121 Khartoum 12702 Alneelain University

Tel: +2499129797

Fax: +24983794422

Abstract:

Background: Previous studies have suggested that erythropoietin (EPO) levels may be inappropriately low in patients with sickle cell disease compared to the extent of the related anemia they demonstrate. Here, we evaluate EPO level vs. renal function, Hb F, and markers of hemolysis for patients with sickle cell disease.

Materials and Method: Blood was drawn from 33 patients with hemoglobin SS aged 5-42 years, during routine visits to the outpatient hematology unit in Military Hospital. Hb F, complete blood count, Erythropoietin level and renal function were measured. The data were analyzed using SPSS 19 and Pearson correlation test was used to find correlation. P value < 0.05 was considered significant.

Results: Neither age nor gender have an effect on EPO level. In addition, a correlation between Hb level and EPO was not consistently observed. Higher EPO levels were seen in patients with high HbS percentage, but no correlation with hemolysis, renal function, or inflammation was observed.

Conclusion: Erythropoietin levels in patients with sickle cell disease do not correlate with known inducers of erythropoietin in healthy individuals.

Introduction:

Homozygous sickle cell Anaemia(SCA) is an autosomal recessive genetic disease that results from the substitution of valine for glutamic acid at position 6 of the β -globin chain, leading to production of haemoglobin S (HbS)(1). Erythropoietin (EPO) is the hematopoietic cytokine that regulates red blood cell production (2).It is produced by peritubular interstitial cells in the renal cortex in response to hypoxia(3). Serum EPO levels are often elevated in chronic anemic states like SCD (4). Though other studies revealed sickled patients produce less EPO at a given hemoglobin concentration than do patients with nonhemoglobinopathy anemia(5-7).This can be explained since sickle hemoglobin has a 'right-shifted oxygen dissociation curve (lower affinity), in which HbS releases more oxygen to tissues at any given oxygen tension than HbA does. Thus, the hypoxia-driven erythropoietin response to anemia may be blunted in steady state SCD (4;8;9). Therefore, relative EPO deficiency could be a contributing factor to the anemia observed in SCA patients(9).

The erythropoietin response in several anemias has been linked to hemoglobin level and tissue hypoxia(10). Though EPO level stimulating factors are not easily understood in SCD patients (11). Since increased red cell production is necessary to maintain given hemoglobin level in patients with a hemolytic disorder like SCD, but it alters EPO/hemoglobin correlations (11). Erythroid hyperplasia may involve a faster clearance of EPO (12). Other factor that complicates EPO response in SCD is the presence of renal damage that is sufficient to decrease renal endocrine function without affecting serum urea and creatinine level (11).

In this study we Here, we evaluate EPO level vs. renal function, HbF level, hemolysis markers and markers of inflammation for patients treated for sickle cell disease.

Materials and Methods:

This was descriptive cross sectional study, conducted from June to November 2014. Blood was drawn from 33 patients with sickle cell anemia during routine visits to the outpatient hematology units, Military hospital-Khartoum-Sudan. Inclusion criteria were: patients homozygous for SCD (SS) as documented by Hemoglobin electrophoreses, aged between 5 and 50 years. Exclusion criteria: patients received blood transfusion within the last three months or admitted to the hospital within 2 weeks because of SCD-related events or crisis. The total number of participants recruited mounted to 33. Five milliliter (ml) of venous blood was collected from each patient, 2.5 ml in(EDTA) container for hematological investigations, measure Hb F level and estimation of plasmaEPO level, and 2.5 ml in lithiumheparin containerfor estimation of LDH. Blood cell counts were performed using automated hematology analyzer "Sysmex". Hemoglobin F was measured by modified fully automated capillary2 flexpiercing hemoglobin electrophoresis technique (Sepia France). LDH was measured by automated chemistry analyzer "Cobas, Integra 400 plus. Plasma was separated from EDTAsample and used for estimation of EPO level by enzyme-linked immunosorbent assay (ELISA) using "Wkea, USA" EPOELISA kit. An ethical clearance was obtained from the Institutional Review Board at Alneelain University. Principal investigator obtained written informed consent from each participant or from parents when the patient was less than 18 years old.

The data were analyzed using SPSS 19 and Pearson correlation test was used to find correlation. P value < 0.05 was considered significant.

Results:

Thirty three patients with HbSS were enrolled in this study 19 (57.6%) of them were males and 14(42.4%) were females; age ranged between 5 and 42years (mean \pm SD: 16.12 \pm 8.9years). Five patients were on a stable dose of hydroxylurea (500mg/day). Erythropoietin level and hematological values are represented in table 1. Gender has no effect on EPO level (P value: 0.972). Age also showed no significant correlation with EPO level (Pearson correlation:-0.22, P.value:0.91). Table 2 indicates the correlations between fetal hemoglobin (HbF), sickle hemoglobin (HbS), hemoglobin, WBCs count, total bilirubin, LDH and EPO level. And correlation was only significant between EPO and HbF and HbS. Linear regression model is illustrated in figure I& II. There was no significant difference in EPO level between patients taking HU and patients who are not (P value: 0.848).

Table I: Hematological and chemistry Baseline Characteristics

Characteristics	Mean ±SD	Median	Maximum	Minimum
H b F (%)	6.15± 5.61	4 . 8	1 7 . 5	0 0
H b S (%)	90.4±5.34	9 2	9 2 . 4	7 9 . 2
H b A ₂ (%)	3.38±0.52	3 . 4	4 . 3	2 . 5
Hemoglobin (g/dL)	7 . 4 ± 1 . 1 2	7	1 1	1 5 . 5
M C V (f L)	83.6±9.37	8 2	1 0 4	7 0 . 7
P C V %	20.82±3.2	20.4	3 1	1 6
M C H (p g)	29.7±3.6	30.6	3 6	2 2
M C H C (g / d L)	35.2±2.22	34.6	4 0 . 3	3 1
Reticulocyte count%	15.58±5.4	16.2	2 6 . 8	6 . 3
Platelets counts (10 ³ /uL)	404.1±125.8	3 8 8	7 2 6	1 8 0
W B C s (10 ³ /uL)	14.36±3.8	1 4	2 3 . 8	7 . 6
Total Bilirubin (mg/dL)	3 . 1 ± 1 . 5	2 . 7	8 . 6	0 . 7
L D H (U / L)	745.79±286.39	6 7 2	1 6 4 2	2 9 8
Erythropoietin (IU/L)	8 . 8 4 ± 3	8 . 5	1 4 . 6	3 . 4

Table 2: Correlation of different biomarkers with EPOlevel among SCA patients

B i o m a r k e r	Person Correlation	P	v a l u e
H b F	- . 3 7 1	0 . 3 4	*
H b S	. 3 6 9	0 . 3 4	*
H b A ₂	. 1 7 2	0 . 3 3	9
H e m o g l o b i n	- . 1 8 6	0 . 3 0	1

W	B	C	s	.	0	3	6	0	.	8	4	2	
Reticulocytes				.	0	1	1	0	.	9	6	6	
L	D	H		0	5	6	0	.	7	5	6		
B i l i r u b i n				0	2	4	0	.	8	9	4		
U	r	e	a	-	0	1	0	0	0	.	5	7	9

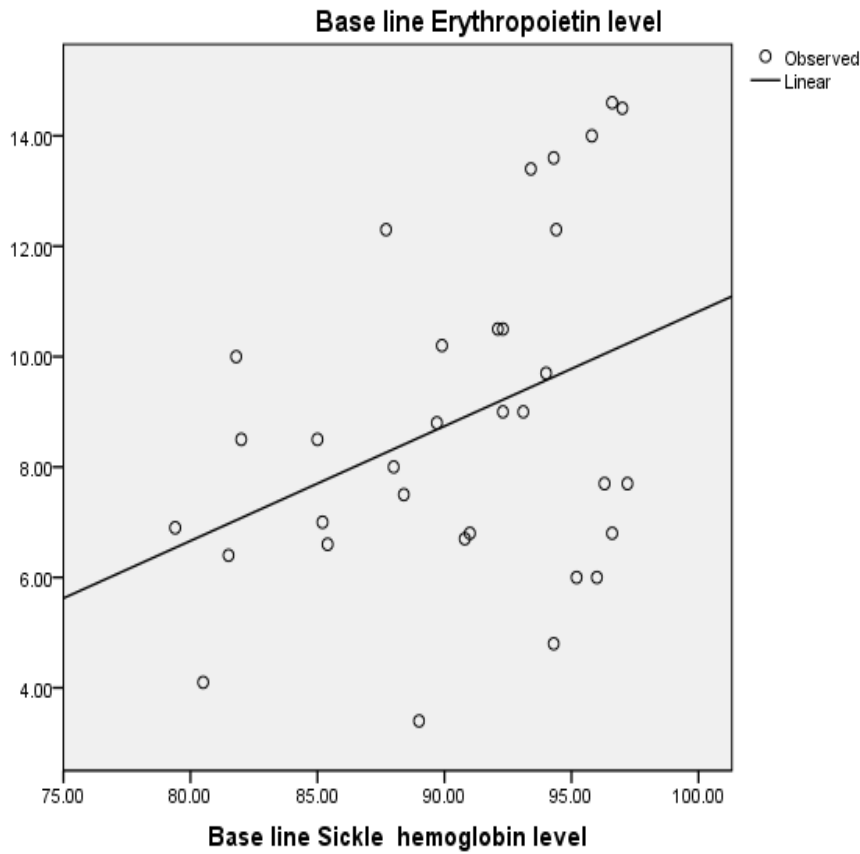


Figure 1: Linear Regression between HbS and EPO level (r^2 : 0.136. P value: 0.34)

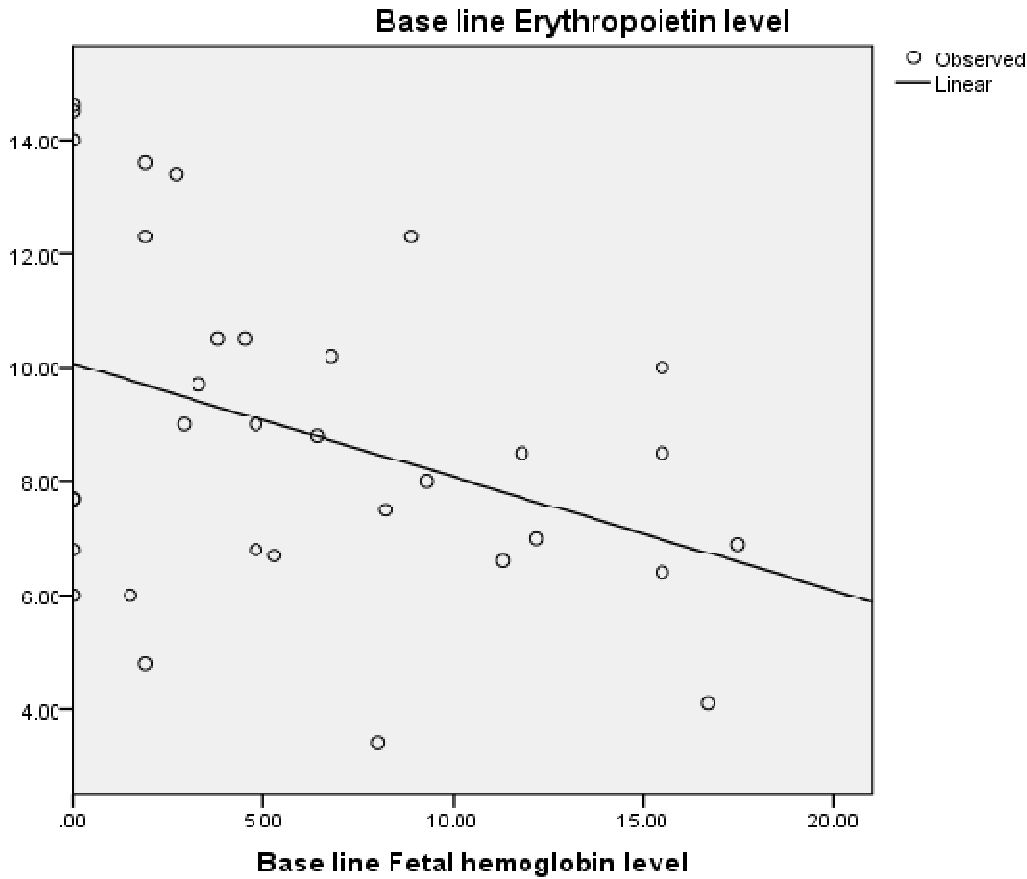


Figure II: Linear

Regression between HbF and EPO level (r^2 : 0.138. P value: 0.34)

Discussion:

The aim of this study was to determine the factors affecting EPO level in sickle cell anemia patients. Our results showed that neither age nor gender influence the plasma level of erythropoietin which is in consistent with previous reviews(10;13). Other studies showed that pediatric sickle cell anemia patients tended to have significantly higher EPO levels than adults(6;14). No correlation between Hb level and EPO was consistently observed (table 2) Pulte *et al* revealed similar results(5). Other investigators revealed negative correlation between hemoglobin and EPO level among SCD patients (7;15). Low EPO among sickle patients was related to renal insufficiency (11;16). However in sickle cell patients in particular, early renal damage may not cause

azotemia but it is sufficient to cause erythropoietin deficiency (11). This could explain why we didn't find correlation between blood urea and EPO level (table 2). Serum EPO level are often elevated in chronic hemolytic anemic states like SCD (4). Though we failed to find any significant correlation between EPO level and hemolysis markers; LDH enzyme, Reticulocyte count% and serum total bilirubin level (table 2). The only factors that appeared to determine plasma EPO level were Hb F and Hb S. We found negative significant correlation with Hb F. Similar result also was documented by Croizat and Nagel who found that low Hb F SCD patients tended to have statistically higher levels of EPO than their high Hb F counterparts (16). Contradictory results were reported by other researchers investigated the relationship between Hb F and EPO level in β thalassemia Intermedia patients (chronic hemolytic anemia) (17). However the same researchers stated that Hb F concentrations above 40% are responsible for greater EPO activity and erythroid expansion (17). Since the highest Hb F percentage in our study is 17.5 % (table 1); this may explain the contradictory results we revealed in our study. We found significant positive correlation between Hb S and EPO level (Fig 2). Since hypoxia is the sole physiologic stimulus for erythropoietin production (13) and Hb S polymerization is mainly determined by low tension of oxygen (18). This may clarify the positive correlation we found between Hb S and EPO level. In spite of the fact that Hb F contributes to a relative tissue hypoxia because of its high affinity for oxygen (19) due to its low affinity for 2,3-diphosphoglycerate (20). This study showed negative correlation between Hb F and EPO, we could interpret these results since Hb F cells have longer survival and more resistance to hemolysis (21). In addition we found significant correlation between Hb F and hemoglobin level (Pearson correlation: 0.450, P value: 0.009). Several studies documented that SCA patients who are receiving HU have higher EPO level than patients who are not (5; 15; 20). In our study HU has no effect on EPO level; this may be due to small dose our patients are receiving per day.

In conclusion, the relationships between anemia, HbF and EPO level in sickle cell anemia are more complex than expected. Further studies and work are necessary to establish the mechanism of the decreased erythropoietin response in the patient with sickle cell anemia.

Acknowledgment:

We would like to acknowledge Dr Omer Eisawi and Dr. Haydar Awad Abdelrazig for significant clinical contribution. We would like to thank Dr Mohamed Ahmed Alfeel and DrYasirAbdeen from Central Laboratory unit Military hospital for their technical assistance in Hb F measurements.Our gratitude to Professor Mustafa Elnimeiri, for his great effort in reviewing and editing the final manuscript.

This research is funded by governmental fund as grant from Higher Education and Scientific Research Ministry.And supporting fund for the principal investigator (LK) from Alneelain University Khartoum Sudan.

Authors' contribution

LK, IF and AS participated in study design and were involved in all aspects of the study conduct. LK, AS, IF, and NE participated in the writing and review of the manuscript. LK and NE analyzed data. LK and NE performed laboratory studies.

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