

Original Research Article

Hb F level among Sickle cell anemia patients

Abstract

Background

Fetal hemoglobin (HbF) can inhibit the deoxygenation-induced polymerization of sickle hemoglobin (Hb S) that drives the pathophysiology of sickle cell disease. The aim of this study was to determine fetal Hb level in Sudanese sickle cell disease patients as well as to find out the association of fetal hemoglobin level on different severity of disease.

Material and Methods

This was descriptive cross sectional study included 100 Patients with sickle cell disease diagnosed by Positive sickling test and Hemoglobin electrophoresis. The Patients were attended Sudan sickle cell anemia center (SSCAC), Elobied-Sudan during September 2015 – July 2016. Clinical history was obtained to perform the severity of the disease according to Hedot et al scoring. Fetal hemoglobin was estimated by Betke's method. Data were analyzed using SPSS software computer program version 21.

Results

The mean of HbF level among the studied population was 7.6%. The descriptive analysis showed that, the mean level of Hb F in 38 (38%) patients with mild disease was 7.7%, while in 54 (54%) patients with moderate disease the mean level of Hb F was 7.6% and the last 8 (8%) patients with severe disease showed Hb F level 7%. There was no statistical significant differences of HbF level in different severity groups in studied patients (P. value = 0.737).

Conclusion

The study concluded that Hb F level has no effect in severity of the disease among studied sickle cell patients.

Key words: sickle cell disease, hemoglobin F, disease severity

Introduction

Sickle cell Anemia (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 hemoglobin S (HbS) [1]. SCA is the most common genetic disorder among people of African descent [2]. In Sudan, SCA is distributed widely in the western region with high prevalence among Messeryi tribe [3].

The clinical state of those patients is exceptionally variable, which may be influenced by different factors, such as the haplotypes of the β^S -globin gene, the presence of alpha-thalassemia, and the fetal hemoglobin level (Hb F level) [4]. Fetal hemoglobin (HbF) plays a dominant role in ameliorating morbidity and mortality of Hemoglobinopathies [5]. HbF level and its distribution among sickle patients is highly variable [6]. Different factors can affect HbF level among SCA patients such as genetic, social, environmental and nutritional factors. Among patients with sickle cell anemia, HbF concentrations vary from 0.1% to 30% with an average of about 8% [7]. High HbF levels may reduce SCA severity due to its ability to inhibit HbS polymerization and also reduce the mean corpuscular HbS concentration [8].

It was been demonstrated that some chemical agents such as placental gonadotropin, progesterone, Azacitidine, Milrinone, erythropoietin, arginine butyrate, phenylbutyrate and hydroxyurea rise hemoglobin level and motivate HbF formation in SCA patients reducing the severity and frequency of SCD episodes [9]. Previous study reported that, HbF level can predict the effect of hydroxyurea treatment, in subjects who start with baseline HbF values between 5 and 10% increases can be two- to three-fold, while subjects with very low baseline HbF can have 10-fold increases post treatment [10]. So estimation of HbF levels may help to devise better therapeutics to induce HbF expression and help clinician to decide the appropriate doses of treatment. On the other hand, determination of Hb F level role in severity of the disease among patients can help in constructing a prognostic model. To our knowledge no previous studies determine HbF level among Sudanese sickle cell patients. The present study was aimed to determine the HbF level among Sudanese sickle cell patients and its effect in different severity condition.

Material and Methods

The study was conducted in 100 sickle cell patients (58% male, 42% female), their age ranged (1 year - 16 years old). All patients were attended to Sudan sickle cell anemia center (SSCAC), El-Obied-Sudan, during September 2015- July 2016. The study had been approved

from the ethical committee of the center, in addition to a written informed consent was obtained from all patients or their parents/caregiver before sample collection.

Structured questionnaire have been designed to collect demographic and clinical data from each patient. The severity score was calculated as described by Hedo et al. (1993)[11], the severity score include Crisis numbers per year, Previous blood transfusion per year, the concentration of Hb and the present or absent of complications such as Pneumonia; Osteomyelitis; Chest syndrome; Heart Failure; Avascular necrosis of femoral head; Renal Failure; Pigment gallstone & Jaundice; Liver Failure; Seizure; Growth retardation; Dehydrated; Acute Splenic Sequestration; VOC/Pain: Generalized or localized. The scoring was classified the patients into mild, moderate or Severe SCA.

A total of 3 ml of venous blood was collected in EDTA anticoagulated tube from each patient, for determination of complete blood count using automated hematology analyzer Sysmex KX-21N[®]. Then, HbF level was estimated by modified Betke's method [12]. In brief, 0.25 ml of hemolysate (10 gm%) was added to 4.75 ml of Drabkin's solution. 0.2 ml of 1.2 NaOH is added to 5.0 ml of resultant HiCN solution and the mixture was gently agitated for 2 minutes. 2 ml of saturated Ammonium Sulphate was added and after shaking, the mixture is allowed to stand for at least 5 minutes. It was then filtered through a double layer of Whatman no.1 filter paper. For the standard, 0.4 ml of HiCN solution, 13.9 ml of water was mixed together. The absorbance of both the test and standard are read using 420 nm filter against water blank. The percentage of HbF was

Calculated as follows:

$$\text{HbF\%} = \frac{\text{Test (Abs)} \times 100}{\text{Std. (Abs)} \times 20}$$

Std. (Abs) x 20

Data were analyzed using SPSS software computer program version 21. Mean of the HbF level among different severity groups patients were determined using independent t-test. The correlation of HbF level in different severity groups' condition was determined using ANOVA test. P-value was considered of significance difference at value of 0.05 and 95% confidence degree.

Results

Over a 10 months period, from September 2015 to July 2016, One hundred patients with sickle cell disease were enrolled in this study. Of them 58 (58%) were males and 42 (42%) were females (M: F ratio of 1.4:1). Their ages ranged from 1– 16 years with a high frequency 65/100 (65%) seen in the age group of patients ranged between 1- 5 years. Most of the

patients had a family history 80/100(80%). The majority of the patients belong to Afro-Asiatic tribes 52/100 (52%), followed by Niger-Congo 48/100 (48%).

The statistical analysis showed that the mean and standard division of HbF level among study population was $7.63 \pm 2.42\%$. It was slightly higher in female than male ($7.74 \pm 2.71\%$, $7.51 \pm 2.05\%$ respectively).

Chi square test showed clinical significant differences of HbF level among age group with P.value = 0.01, table (1).

Table 1: Hemoglobin F levels in different age groups

Age groups (years)	Frequency	Mean of HbF level ($\pm$ SD)	P.value
1 – 5 yrs	59	5.40 ± 2.31	0.001
6 – 10 yrs	23	7.80 ± 2.48	
11 – 16 yrs	18	9.55 ± 2.77	

The severity of disease was variable in the study population when Hedo scoring was applied; 38 (38%) of the patients was presented with mild feature, while 54 (54%) of the patients presented with moderate disease and only 8 (8%) patients had severe.

The mean of HbF level in patients with mild disease severity was $7.7 \pm 2.82\%$ ranging between (2.1% - 13.4%), further more in patients with moderate disease severity the mean of HbF level was $7.65 \pm 2.23\%$ the range was (3.2% - 12.1%), and in severe case the mean was $7.00 \pm 1.51\%$ range was (4% - 10%). There was no clinical significant differences of HbF level in different severity groups of the patients (P. value = 0.737) Table 2.

Table 2: Hb F level in different Severity group of study patients

Degree of Severity	Mean $\pm$ SD	P. value
Mild	7.7 $\pm$ 2.8	
Moderate	7.6 $\pm$ 2.2	
Severe	7 $\pm$ 1.5	

A total of 71/100 (71%) of the patients showed Hb F level < 10%, while the rest 29/100 (29%) was showed HbF $\geq$ 10%. Of them 19/29 (65.5%) were Female and 10/29 (34.5%) were male. The disease severity of the 29 patients was showed in table 3, of them 15/29 (51.7%) showed moderate disease, while 13/29 (44.8%) presented with a mild disease and only one patients (3.5%) was showed severe disease. The goodness of fit test using Chi square, showed that there is a significant differences of disease severity when HbF level $\geq$ 10% with P.value = 0.03.

Table 3: Severity of the disease in patients with HbF $\geq$ 10%

Hb F %	Gender	Mild disease	Moderate disease	Severe disease	P. Value
10%	Female	1	7	1	0.03
	Male	5	3	-	
11%	Female	5	5	-	
	Male	1	-	-	
13%	Female	-	-	-	
	Male	1	-	-	

Discussion

The heterogeneous phenotype in patients with sickle cell disease (SCD) is determined by the interaction of genetic and environmental factors. One of them is Hb F level which influenced on disease manifestation in many studies [13]. The objective of our study was to determine Hb F level and examine the relationship between HbF level and disease severity among

Sudanese patients with SCA attending Sudan sickle cell anemia center (SSCAC), Northern Kordofan State, Western Sudan.

The average HbF level in our population was 7.6% which is lower comparable to other studies in Congo [14], Nigeria [15], and Saudi Arabia [16] where the mean Hb F levels were 8.8%, 9.5% and 9.1% respectively.

The study found that the mean of Hb F level among patients study was slightly higher in female than male, this finding was disagree with study done in Nigeria by Kotila et al, where males recorded a higher HbF levels than females, although the study in Nigeria was done among adults [17]. In the other hand, the study showed there is a difference of HbF level according to the age group. Higher levels were found in older age. Many study showed that genetic loci are known to increase HbF levels in adult life [18]. Our study confirmed earlier findings that HbF level is higher in sickle cell adults [19].

One important factor that has been described as influencing the clinical course and hence disease severity in SCA is HbF level [20]. Populations with high HbF levels like those in Eastern Saudi Arabia have been described as having less severe disease with fewer complications [21] and better survival [22]. Our study showed that the mean of Hb F level in patients with a severe disease was 7%, in patients with a moderate disease was 7.6% and in patients with a mild disease severity was 7.8%. there was no statistical significant differences of HbF level in different severity groups of the patients, Our study was in accordance with study done by Lena Mpalampa et al [23], and Konotey et al [24] they showed that some sicklers had frequent crisis and their HbF levels were below 20%, and those with mild crisis had HbF levels above 20%. Middle East sicklers who have frequent crisis were also found with 30% HbF [24].

One of our study observation was that, Hb F level play a role in a disease severity when it is more than 10% as showed in 29 patients, denotes that HbF level more than 10% represent a higher level with a less severity, this in accordance with many study mentioned high HbF level had no or mild crisis (25)

Conclusion

The study concluded that Hb F level has no effect in severity of the disease among studied sickle cell patients, unless the HbF level more than 10%.

References

- 1- Fathallah H, Atweh GF. Induction of fetal hemoglobin in the treatment of sickle cell disease. Hematology Am Soc Hematol Educ Program 2006;58–62.
- 2- Silva-Pinto AC, Angulo IL, Brunetta DM, Neves FI, Bassi SC, Santis GC, et al. Clinical and hematological effects of hydroxyurea therapy in sickle cell patients: A single-center experience in Brazil. Sao Paulo Med J. 2013;131(4):238–43.
- 3- Sabahelzain MM, Hamamy H. The ethnic distribution of sickle cell disease in Sudan. Pan Afr Med J 2014;18:13
- 4- Steinberg MH. Predicting clinical severity in sickle cell anaemia. Br J Haematol. 2005;129(4):465–81
- 5- Moumni I, BenMustapha M, Ben Manour I et al. Fetal Hemoglobin In Tunisian Sickle cell disease patients: Relationship with polymorphic sequences Cis to the B-Globin Gene. Indian J Hematol Blood Transfus, 2016;32(1):114-9
- 6- Chaouch L, Moumni I, Ouragini H et al. rs11886868 and rs4671393 of BCL11A Association with HbF level variation and Modulate clinical events among sickle cell anemia patients. Hematology. 2016;22: 1-5.
- 7- Ofori-Acquah, S.F., Lalloz, M.R., Serjeant, G. & Layton, D.M. (2004) Dominant influence of gamma-globin promoter polymorphisms on fetal haemoglobin expression in sickle cell disease. Cellular and Molecular Biology, 50, 35–42.
- 8- G. Lettre, The search for genetic modifiers of disease severity in the hemoglobinopathies, Cold Spring Harb. Perspect. Med. 2 (10) (2012) 1–12
- 9- Green, N. S., & Barral, S. (2014). Emerging science of hydroxyurea therapy for pediatric sickle cell disease. *Pediatr Res*, 75(1-2), 196-204.
- 10- Steinberg MH, Voskaridou E, Kutlar A, et al. Concordant fetal hemoglobin response to hydroxyurea in siblings with sickle cell disease. Am J Hematol 2003;72:121–126.
- 11- Hedo C.C., Aken'ova Y.A., Okpala I.E., Durojaiye A.O., Salimonu L.S. (1993). Acute phase reactants and severity of homozygous sickle cell disease. Journal of Internal Medicine 233: 467–470.
- 12- Prehu C, Ducrocq R, Godart C, Riou J, Galacteros F: Determination of Hb F Levels: The Routine Methods. Hemoglobin 1998, 22:459–467.
- 13- Sebastiani P, Nolan VG, Baldwin CT, Bad-Grau MM, Wang L, Adewoye AH, et al. A network model to predict the risk of death in sickle cell disease. Blood 2007 Oct 1;110(7):2727-35.
- 14- Mouele R, Galacteros F, Feingold J: Haemoglobin F (HbF) levels in sickle-cell anaemia patients homozygous for the Bantu haplotype. Eur J Haematol 1999, 63:136–137.
- 15- Fatunde OJ, Scott-Emuakpor AB: Haemoglobin F and A2 in Nigerian children with sickle cell anaemia. J Trop Pediatr 1993, 39:251–252.

- 16- El-Hazmi MAF: Clinical and Haematological Diversity of Sickle cell disease in Saudi Children. *J Trop Pediatr* 1992, 38:106–112.
- 17- Kotila Tr, Fawde OI, Shokunbi WA. Haemoglobin F and clinical severity of sickle cell anaemia among Nigerian adults. *Afr J Med Sci*. 2000 Sep –Dec; 29 (3-4): 229 – 31.
- 18- Thein SL, Sampietro M, Rohde K, Rochette J, Weatherall DJ, Lathrop GM, Demenais F. Detection of major gene for hetrocellular hereditary persistence of foetalhaemoglobin after accounting for genetic modifiers. *American Journal of Human Genetics*. 1994;54:241–228.
- 19- Dominic Edoh, Charles Antwi- Bosaiko, and Dominic Amuzu Fetal hemoglobin during infancy and in sickle cell adults *Afr Health Sci*. 2006; 6(1): 51–54.
- 20- Charache S: Fetal hemoglobin, sickling, and sickle cell disease. *Adv Pediatrics* 1990, 37:1–31.
- 21- Perrine RP, Pembrey ME, John P, Perrine S, Shoup F: Natural History of Sickle Cell Anemia in Saudi Arabs; A Study of 270 Subjects. *Ann Intern Med* 1978, 88:1–6.
- 22- Platt OS, Brambilla DJ, Rosse WF, Milner PF, Castro O, Steinberg MH, Klug PP: Mortality In Sickle Cell Disease – Life Expectancy and Risk Factors for Early Death. *N Engl J Med* 1994, 330:1639–1644.
- 23- Lena Mpalampa, Christopher M Ndugwa, Henry Ddungu and Richard Idro ,Foetalhaemoglobin and disease severity in sickle cell anaemia patients in Kampala, Uganda, *BMC Blood Disorders* 2012, 12:11
- 24- KonoteyAhulu FID. The sickle cell disease patient. 1991:91–108.
- 25- Omoti CE. The value of foetal hemoglobin level in the management of Nigerian sickle cell aneamia patients. *Nigeria Postgraduate Medical Journal*. 2005;12(3):149–154.