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The effect of fetal hemoglobin on RBC parameters among sickle cell anemia patients: a cross sectional study from Makkah city; Western Saudi Arabia

Abstract

Background: Sickle Cell Anemia (SCA) is the most common monogenic disorder that is inherited as an autosomal recessive pattern. Fetal hemoglobin (HbF) plays a major role in ameliorating clinical severity of the SCA where higher expression of HbF is associated with a reduction in the painful episode and thus reduces the number of hospitalization. The current work aimed to investigate the effect of high HbF in SCA adult patients in Makkah city, Western Saudi region who visited Al-Noor Specialist Hospital.

Methodology: Blood samples in EDTA tubes were collected from 80 SCA adults patients during the period of February, 2015 to April, 2016. Hematological analyses including Complete Blood Cell Count (CBC) and reticulocyte count were performed to assess anemia status. Hemoglobin quantification and separation was performed using High Pressure Liquid Chromatography (HPLC). Patients with hydroxyl urea intake were excluded from the study due to its elevation effect on HbF level.

Result: Of the 80 studied patients, 60(75%) showed an increased level of HbF above the upper normal limit with a mean of 6.01% +/-3.5. The increase level of HbF was more than 10%, 5-10%, and less than 5% in 6(10%), 24(40%) and 30(50%) patients respectively. A significant association between high HbF and red cell parameters was observed in patients with higher HbF than 10% compared to the other two groups.

Conclusion: High HbF gives an advantage to the SCA patients where it has an ameliorating effect on the severity and the complications of the disease. The elevated HbF in Makkah city is high among adult SCA patients (75%) but the average expression level is lower than other Saudi regions. This indicates patients from Makkah City have more clinical severity and thus highly attended healthcare is required to minimize disease effect and its complication. Further molecular studies are recommended to find out genetic determinants such as single nucleotide polymorphisms of high HbF expression in SCA.

Keywords: sickle cell anemia, hemoglobin F, reticulocyte count, red blood cell indices

Volume 3 Issue 1 - 2016

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Received: October 01, 2016 | **Published:** November 21, 2016

Abbreviations: SCA, sickle cell anemia; HbS, hemoglobin S; SCT, sickle cell trait; HbF, hemoglobin F; CBC, complete blood count; HPLC, high performance liquid chromatography; ARC, absolute reticulocyte count

Introduction

Sickle cell Anemia (SCA) is the most common monogenic disorder in the world with an estimation of 210,000 infants being affected every year.[1] It is inherited as an autosomal recessive pattern and characterized by production of sickle hemoglobin (HbS) that results from a single nucleotide change in the β-globin gene causing a substitution of valine for glutamic acid at position 6 of the β-globin chain ($\beta^{6glu\rightarrow val}$ or β^{s}).[2] In Saudi Arabia, about 4.2% of the population is found as Sickle Cell Trait (SCT) and 0.26% has SCA.[3] Most of the patients live in the Eastern and Southwestern regions and the highest prevalence reported in the Eastern region where 17% of the population is sickle cell trait and 1.2% is SCA.[3] The prevalence of the disease in other Saudi regions including Central and Western regions was estimated to be 0.06 and 0.12% respectively.[4] The clinical severity of the disease varies greatly from asymptomatic to severe hemolytic anemia that requires regular blood transfusion. Several factors can ameliorate the clinical severity of the SCA. These include fetal hemoglobin (HbF), coinheritance of alpha thalassemia and modifying or epistatic genes such as mediators of inflammation, oxidant injury, growth factors, and transcriptional regulators.[5] The level of HbF ($\alpha 2\gamma 2$) plays a major role in reducing clinical symptoms and acts as a prognostic factor for the sickler patients because it interferes with the polymerization and crystallization of HbS resulting in decreasing the hemolytic episodes.[6] The HbF level is variably expressed depending on the beta S-globin haplotypes, which reflect the ethnic background of the SCA patients. Several studies have identified these genetic determinants as single nucleotide polymorphisms that are found at several loci and associated with variable amounts of HbF in patients with SCA or β-thalassemia and in healthy adults. These loci are responsible for 20 to 50% of the HbF trait variance in patients with β-thalassemia or sickle cell disease and in healthy Europeans.[7–10] In Saudi Arabia, most of the Eastern region patients carry the Saudi-Indian β-globin

gene-like cluster haplotype with high HbF that is characterized with a mild course of the disease.[11] On the other hand, the Southwestern region SCA patients have African-derived haplotype with the lower HbF level and severe symptoms.[12] Up to our knowledge, no previous studies have been done about the level of high HbF in SCA patients in Makkah city, Western region. Therefore, the aim of this study was to estimate high HbF in the SCA adult patients from Makkah city and analyze its association with red blood cell parameters and reticulocyte count.

Methods

Patient recruitment and samples collection

This was a cross sectional study performed on SCA adults patients (ages ranged from 18 to 52years) visited Al-Noor Specialist Hospital in Makkah city, Western region over 1year period from February, 2015 to April, 2016. Eighty patients (Male=41, Female=39) were recruited and blood samples were collected after signing consent forms. Patients on Hydroxy Urea (HU) treatment were excluded from the study due to its effect on HbF level. In order to determine the normal range of HbF level in our population, 50 blood samples from healthy individuals were also collected.

Hematological Analysis

Complete Blood Cell Count (CBC) including RBC count, Hemoglobin concentration, hematocrit, RBC indices, red cell distribution width and reticulocyte with its absolute count were performed on sickler and normal samples using XN-1000™ Hematology Analyzer (Sysmex, Lincolnshire, USA). Hemoglobin separation and quantification were performed by High Performance Liquid Chromatography (HPLC) technique using VARIANT™ II Hemoglobin Testing System (Bio-Rad Laboratories, Hercules, CA, USA). The relationship between HbF level and red blood cell parameters for the SCA patients was assessed by dividing the patients into three groups depending on the HbF level: 2.5-5%, 5-10% and >15%.

Statistical analysis

All statistical analyses were carried out using the Statistical Packages for Social Sciences (SPSS Inc., Chicago, IL, USA) software version 23.0. Data were expressed as range and mean± Standard Deviation (SD). The association study was achieved by using Pearson correlation coefficient test, while the comparisons between groups were done by using one-way analysis of variance (ANOVA). For all quantitative variables, P<0.05 was considered as statistically significant.

Results

Over 1 year period, February-2015 to April-2016, 80 SCA adult patients were recruited and enrolled for this study in Al-Noor Specialist Hospital. 60 (75%) patients out of 80 showed a mean level of HbF 6.01%+/-3.5 above the upper normal limit (Figure 1) with no significant difference between male and female (p=0.35). The level of HbF was more than 10%, 5-10%, and less than 5% in 6(10%), 24(40%) and 30(50%) patients respectively (Table 1). A significant association between high HbF and red cell parameters was observed in patients with higher HbF than 10% (p value <0.05) compared to the other two groups indicating a positive association. There was a direct relationship between the level of HbF and the average Hb concentration; the more HbF level the closer to the normal Hb concentration. The relationship between HbF level and other red cell parameters including Hct and red cell indices did not show significant difference except for the patients group of >10% HbF. Absolute Reticulocyte Count (ARC) was also measured to evaluate if it can be used as an indicator for the level of HbF and the result showed there was statistically significant difference between HbF and ARC. The higher HbF is associated with the lower ARC and patients in >10% HbF showed lesser ARC compared to the other two groups.

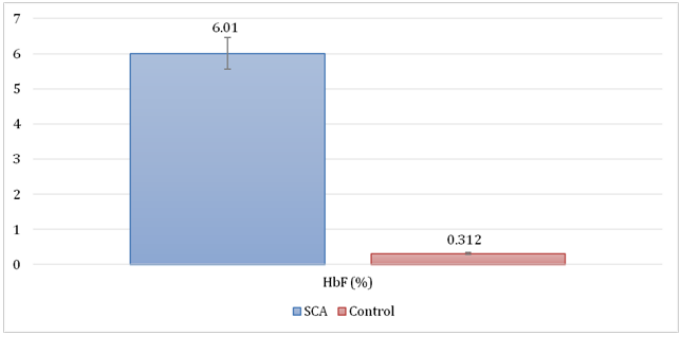

Figure 1 The mean of HbF level in SCA patients and normal control.

Table 1 The relationship between high HbF and red cell parameters in adult patients of SCA

RBC parameters	Normal ranges	HbF Level					
		2.1-4.9% (N=30; 50%)	P-value	5-10% (N=24; 40%)	P-value	>10% (N=6; 10%)	P-value
Hb(g/dl)	Male:13-17 Female:12-15	8.9±2.2	0.066	8.5±1.6	0.671	8.9±0.7	<0.05
Hct(%)	40-50	26.7±7.6	0.053	24.9±4.7	0.713	25.9±3	<0.05
MCV (fl)	80-100	82.5±9.7	0.136	81.64±11.8	0.189	80.2±11.1	<0.05
MCH (pg)	27-32	28.1±4	0.174	28.2±5	0.096	27.9±4.8	<0.05
MCHC(g/dl)	31.5-34	33.7±1.5	0.171	33.7±2.1	0.001	35.6±1.6	<0.05
ARC(x10^6/µl)	0.017-0.064	2.11±8.8	<0.05	0.3±0.1	<0.05	0.2±0.04	<0.05
Male		17		13		1	
Female		13		11		5	

Citation: Khojah A, Faidah H, Sami I, et al. The effect of fetal hemoglobin on RBC parameters among sickle cell anemia patients: a cross sectional study from Makkah city;Western Saudi Arabia. *Hematol Transfus Int J.* 2016;3(1):141–145. DOI: 10.15406/htij.2016.03.00059

In order to detect if there is a relationship between high HbF and increase in the age, we categorized patients with high HbF into five groups namely <20, 20-30, 31-40 and >40 (Table 2). Our results showed that the highest level of HbF was observed in young adults under 20 years followed by patients between 31-40, 20-30 and >40years with (6.22%±3.3), 6.05±3.4, 5.97±3.8 and 4.83±2 respectively. This means there is no correlation between HbF levels with increased age of the SCA patients. SCA is typically a normochromic normocytic type of anemia. However, during our investigation, it was noted that a significant number of patients (27/80-33.75%) showed microcytic anemia among normal HbF and high HbF (Table 3). This indicates the possibility of presence of microcyosis causing elements such as iron deficiency anemia or thalassemia which is commonly found in our area and might be co-inherited with the sickling allele. The presence of iron deficiency as a causative element was ruled out by normal ferritin level of the identified subjects. More than half of the patients with normal HbF (55%) showed microcytosis among patients with normal HbF level while it was 26.7% in patients with high HbF. The anemia status in patients with normal HbF was worse than microcytic group while it was almost same in patients with high HbF. In addition, both groups showed moderate microcytosis but more in patients with high HbF level.

Table 2 Frequency of high HbF among age groups

Age	Number of patients	Mean HbF (%)
<20	18	6.22±3.3
20-30	28	5.97±3.8
31-40	10	6.05±3.4
>40	4	4.83±2
Total	60	6.01±2

Table 3 Comparison between the hematological parameters of SCA patients with normal versus high HbF level

RBC parameters	Normal range	Normal HbF		High HbF	
		Normocytic RBCs	Microcytic RBCs	Normocytic RBCs	Microcytic RBCs
RBC(x10⁶/µl)	Male: 5-6.1 Female: 4.2-5.2	2.8±0.5	3.8±0.9	2.9±0.8	3.9±0.7
Hb(g/dl)	Male: 13-17 Female: 12-15	7.4±1.1	9.4±2.2	8.8±2.1	8.7±1.1
Hct (%)	40-50	21.4±3.8	28±5.7	25.7±7	26.5±3.5
MCV(fl)	83-101	87.1±5.9	72.1±4.8	87.4±5.6	67±5
MCH(pg)	27-32	30.6±3.2	24.1±2.3	30.3±2.8	22.2±2
MCHC(g/dl)	31.5-34	35±1.9	33.5±1.8	34.1±1.9	33±1
RDW (%)	11.6-14	21.7±4.2	21.7±4.5	20.1±3.9	20.4±3.9
ARC(x10⁶/µl)	0.017-0.064	0.4±0.3	0.32±0.31	0.3±0.2	0.1±0.0
Total Number		9(45%) 20(100%)	11(55%)	44(73.3%) 60(100%)	16(26.7%)

Discussion

SCA is among the most challenging disorders worldwide due to its impact on healthcare that require highly attended and effective programs to control and eliminate such disorder. Since there is no comprehensive care of SCA patients, the health care utilization cost is going to be increased.[13] In Saudi Arabia, the prevalence of SCD is quite common due to consanguineous marriage that exceeds 50%.[14] The first reported HbS gene was in Eastern province and since then the southern province had been documented to have HbS gene in both heterozygous and homozygous forms.[15,16] The variation of the clinical symptoms in the SCA patients is related to the genetic makeup. In other words, the clinical features of the SCA patients from the western province are linked with the influence of beta-globin gene haplotype and the origin of the sickle cell gene.[17] Unfortunately, our lab does not have molecular diagnostic and research facilities to identify the beta-globin gene haplotype of our samples study. However, one of the ameliorating factors that affect clinical severity of the SCA is the high level of HbF where it is linked to the milder form of SCA. Makkah city is one of the main cities in the Western province of Saudi Arabia and considered as the holy capital city of the Islamic world because of the holy mosque. It is a multicultural city and many of its residents with the Saudi citizenship descended from different genetic background such as African, Southeast Asia and Middle East countries. There is no clear picture about the estimation of high HbF level among SCA patients in Makkah city and thus the focus of this study was to investigate the effect of high level of HbF in the SCA patients and its relationship with the red cell parameters and compare its level with other Saudi regions and cities.

Our results showed that 75% of the enrolled adult patients (60/80) had an increase in the HbF level with an average 6.01%±3.5. The influence of HbF level on SCA is well known to ameliorate disease morbidity and mortality in adulthood based on clinical observation. Therefore, the higher expression of HbF reduces disease severity in term of disease crisis and complications. A comparative study performed in children with SCA from different Saudi regions showed that the average HbF level in Makkah was 5.5±3.9.[18] Our investigation in adult patients showed almost a similarity in the HbF level between our study and the study performed in the children by El-Hazmi et al.[18] Another study performed in the Eastern province showed that SCA patients in this region had a mean HbF level of 25.9% making them to have the mildest form of the disease due to the HbF ameliorating effect.[11] Comparing our results with these two studies, we can infer

that SCA patients with high HbF in Makkah city have a clinical severity more than Eastern and Southwestern regions and patients' care should be given more attention to minimize its effect on disease complications.

The relationship between high HbF and red cell parameters was also investigated (Table 1) and our results showed that the average value of the Hb concentration ranged from 8.5-8.9 with an average normal RBC indices indicating normochromic normocytic type of anemia. This is a typical feature of SCA with HbSS genotype and is consistent with the results of other studies.[18–20] However, a significant number of patients showed moderate microcytosis among all studied SCA wither with normal or high HbF comprising 55% and 26.7% respectively (Table 3). This is not unusual since other factors such as iron deficiency anemia and inheritance of thalassemia element may alter morphological classification of the SCA. Presence of iron deficiency as a causative element of microcytosis in the studied subjects was ruled out by the level of serum ferritin which was found normal. Thalassemia is commonly found in Saudi Arabia particularly in the Eastern province where high frequency of α- and β-thalassemia was reported.[21,22] In our case, we anticipated that our patients may have a co-inheritance element of thalassemia trait due to the moderate microcytosis and molecular studies of α- and β-globin genes can warrant the causative element of the moderate microcytosis observed in the patients. Unfortunately, we were unable to conduct such analysis due to unavailability of molecular diagnostic tests in the hospital and limited budget. In addition, the level of HbA2 cannot be used as a diagnostic parameter for the presence of thalassemia trait due to co-elution of glycated HbS in the same position of HbA2.[23]

In order to evaluate the effect of high HbF on the degree of hemolysis, we measured the ARC in all patients with normal and high HbF to observe if ARC can be used as an indicator for the level of HbF. We found that there was a statistically significant difference between HbF and ARC. The higher HbF is associated with the lower ARC and patients in >10% HbF group showed reduced ARC compared to the other two groups (Table 1). This indicates the advantage effect of increasing HbF to maintain the hemolysis rate at minimal degree. Our finding is consistent with a recent study performed on SCA patients with increased HbF which showed reduced reticulocyte count and lactate dehydrogenase while patients with low HbF level showed increased ARC.[24]

Conclusion

In conclusion, high HbF gives an advantage to the SCA patients where it has an ameliorating effect on the severity and the complications of the disease. The average expression level is lower than other Saudi regions including Eastern and Southwestern regions where the expression level of elevated HbF was 25.9% and 10% respectively. This indicates patients from Makkah City have more clinical severity and thus highly attended healthcare is required to minimize disease effect and its complication. Further molecular studies are recommended to find out genetic determinants such as single nucleotide polymorphisms of high HbF expression in SCA.

Acknowledgements

This study was approved by human medical ethics at Al-Noor Specialist Hospital (Letter No. 61147). The authors would like to thank Mr. Ahmed Ghouth and Mr. Naif Al Abidi for their assistance in collecting blood samples.

Conflict of interest

The author declares no conflict of interest.

References

1. Bhaskar P Urade. Incidence of sickle cell anaemia and thalassaemia in central India. *OJBD*. 2012;2(4):71–80.
2. Daniel Catovsky, Edward GD. *Postgraduate Haematology*. In: Hoffbrand AV, et al. editors. John Wiley and Sons. 6th ed. USA: Blackwell Publishing; 2011. p. 1–1074.
3. Jastaniah W. Epidemiology of sickle cell disease in Saudi Arabia. *Ann Saudi Med*. 2011;31(3):289–293.
4. Al-Qurashi MM, El-Mouzan MI, Al-Herbish AS, et al. The prevalence of sickle cell disease in Saudi children and adolescents. A community-based survey. *Saudi Med J*. 2009;29(10):1480–1483.
5. Steinberg MH. Predicting clinical severity in sickle cell anaemia. *Br J Haematol*. 2005;129(4):465–481.
6. Makani J, Williams TN, Marsh K. Sickle cell disease in Africa: burden and research priorities. *Ann Trop Med Parasitol*. 2007;101(1):3–14.
7. Fanis P, Kousiappa I, Phylactides M, et al. Genotyping of BCL11A and HBS1L-MYB SNPs associated with fetal haemoglobin levels: a SNaPshot minisequencing approach. *BMC Genomics*. 2014;15:108.
8. Lettre G, Sankaran VG, Bezerra MA, et al. DNA polymorphisms at the BCL11A, HBS1L-MYB and beta-globin loci associate with fetal hemoglobin levels and pain crises in sickle cell disease. *Proc Natl Acad Sci U S A*. 2008;105(33):11869–11874.
9. Garner C, Tatu T, Reittie JE, et al. Genetic influences on F cells and other hematologic variables: a twin heritability study. *Blood*. 2000;95(1):342–346.
10. Danjou F1, Anni F, Perseu L, et al. Genetic modifiers of beta-thalassemia and clinical severity as assessed by age at first transfusion. *Haematologica*. 2012;97(7):989–993.
11. Alabdulaali MK. Sickle cell disease patients in eastern province of Saudi Arabia suffer less severe acute chest syndrome than patients with African haplotypes. *Ann Thorac Med*. 2007;2(4):158–162.
12. Alsultan A, Aleem A, Ghabbour H, et al. Sickle cell disease subphenotypes in patients from Southwestern Province of Saudi Arabia. *J Pediatr Hematol Oncol*. 2012;34(2):79–84.
13. Iughetti L, Bigi E, Venturelli D. Novel insights in the management of sickle cell disease in childhood. *World J Clin Pediatr*. 2016;5(1):25–34.
14. El Mouzan MI, Al Salloum AA, Al Herbish AS, et al. Consanguinity and major genetic disorders in Saudi children: a community-based cross-sectional study. *Ann Saudi Med*. 2008;28(3):169–173.
15. Atweh GF, DeSimone J, Sauntharajah Y, et al. Hemoglobinopathies. *Hematology Am Soc Hematol Educ Program*. 2003;2003:14–39.
16. El-Hazmi MA, Warsy AS, Al-Swailem AR, et al. Sickle cell gene in the population of Saudi Arabia. *Hemoglobin*. 1996;20(3):187–198.
17. El-Hazmi MA, Warsy AS. Appraisal of sickle-cell and thalassaemia genes in Saudi Arabia. *East Mediterr Health J*. 1999;5(6):1147–1153.
18. El-Hazmi MA, Warsy AS. A comparative study of haematological parameters in children suffering from sickle cell anaemia (SCA) from different regions of Saudi Arabia. *J Trop Pediatr*. 2001;47(3):136–141.
19. Cavalcante JE, Machado RP, Laurentino MR, et al. Clinical events and their relation to the tumor necrosis factor-alpha and interleukin-10 genotypes in Sickle-Cell-Anemia patients. *Hematol Oncol Stem Cell Ther*. 2016;9(1):14–19.

20. Akinbami A, Dosunmu A, Adediran A, et al. Haematological values in homozygous sickle cell disease in steady state and haemoglobin phenotypes AA controls in Lagos, Nigeria. *BMC Res Notes*. 2012;5:396.

21. Al-Ali AK, Al-Ateeq S, Imamwerdi BW, et al. Molecular bases of beta-thalassemia in the Eastern Province of Saudi Arabia. *J Biomed Biotechnol*. 2005;2005(4):322–325.

22. Al-Awamy BH. Thalassemia syndromes in Saudi Arabia. Meta-analysis of local studies. *Saudi Medical Journal*. 2000;21(1):8–17.

23. Craver RD, Abermanis JG, Warrier RP, et al. Hemoglobin A2 levels in healthy persons, sickle cell disease, sickle cell trait, and beta-thalassemia by capillary isoelectric focusing. *Am J Clin Pathol*. 1997;107(1):88–91.

24. Moreira JA, Laurentino MR, Machado RP, et al. Pattern of hemolysis parameters and association with fetal hemoglobin in sickle cell anemia patients in steady state. *Rev Bras Hematol Hemoter*. 2015;37(3):167–171.

Citation: Khojah A, Faidah H, Sami I, et al. The effect of fetal hemoglobin on RBC parameters among sickle cell anemia patients: a cross sectional study from Makkah city;Western Saudi Arabia. *Hematol Transfus Int J.* 2016;3(1):141–145. DOI: 10.15406/htij.2016.03.00059