Original Article

Haemoglobin F and A_2 profiles among Sickle Cell Anaemia Patients in Lagos State University Teaching Hospital (LASUTH), Nigeria

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Abstract

Background: The choice of high-performance liquid chromatography (HPLC) to measure HbF and HbA_2 in sickle cell disease patients is regarded as a method of choice by many researchers. This study was aimed at using HPLC in determining the mean and gender-specific reference values of HbF and HbA_2 in sickle cell anemia (SCA) population and bringing to fore all associated implications. **Materials and Methods:** This was a cross-sectional, retrospective, descriptive study involving SCA patients. All case notes containing HPLC hemoglobin quantification reports were reviewed to extract the percentages of HbA_2, HbF, and HbS of patients. The demographic data of individual patients were also obtained from the records. Data were analyzed with IBM SPSS Statistics for Windows, Version 20.0 Armonk, New York, USA. **Results:** A total of 100 participants' records were reviewed consisting of 40 (40%) males and 60 (60%) females. The overall mean age (±standard deviation [SD]) of participants was 25.89 years ±9.34. The overall mean HbF and HbA_2 were 6.94% ±5.05 and 3.75% ±0.74, respectively. Thirty percent had HbF <3%, whereas 34% of them had elevated HbA_2 level >4%. The mean (±SD) HbF and HbA_2 for both males and females were 6.97% ±5.45 and 3.68% ±0.58, 6.92% ±4.87, and 3.80% ±0.83, respectively. **Conclusions:** Thirty percent of the study participants had HbF <3%, whereas 34% of them had elevated HbA_2 level >4% and could indeed be carrying beta thalassemia trait with the sickle cell gene.

Keywords: Hemoglobin profiles, HbA2, HbF, high-performance liquid chromatography, sickle cell anemia

Introduction

Sickle cell anemia (SCA) is common in Nigeria with prevalence values ranging from 2% to 3% of the 140 million populations.[1]

SCA is characterized by an abnormal hemoglobin structure consequent on replacement of adenine with thymine on the β-globin gene resulting in valine replacing glutamic acid on the β chain of the hemoglobin structure.[2] Co-inheritance of α-thalassemia gene with SCA causes a reduction in mean corpuscular hemoglobin concentration which inhibits hemoglobin S polymerization, thus causing an increase in hemoglobin levels which may increase blood viscosity in SCA.[3] Conversely, co-inheritance of β thalassemia gene with SCD impacts negatively on disease severity.

The World Health Organization recognized SCA as a global public health problem in 2006.[1] It also included SCA in 2010 Global Burden of Diseases, Injuries and Risk factors.[4,5] The sickle cell trait (HbAS), individuals who are carriers of the disease, have a normal hemoglobin and a sickled hemoglobin, they are usually asymptomatic and the prevalence ranges from 10% to 40% in sub-Saharan Africa.[6]

SCA is the symptomatic presentation of the inherited mutation which can present depending on inheritance as severe which is SCA in which the inheritance is HbSS or HbSβ thalassemia, HbSS is seen in 2%–3% of Nigerians.[7,8]

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Hemoglobin structure

The functional properties of hemoglobin are determined by their characteristics folds of amino acid chains of the globin proteins including seven stretches of the polypeptide α helix in the α chains and eight β helix in the β chains.[9] The reversible binding of O_2, CO, and NO to the four ferrous iron atoms of the heme is responsible for transportation of these gases by hemoglobin.[10]

Hemoglobin phenotypes

Normal adult hemoglobin structure of α_2 β_2 is known as hemoglobin A, it accounts for 97% of total hemoglobin. Other hemoglobin types present in adult are hemoglobin A_2 which is α_2 δ_2. It accounts for 2% and hemoglobin F α_2 γ_2 which accounts for 1% of adult hemoglobin.[11]

Hb F is the best-suited hemoglobin in-utero because it has a slightly higher oxygen affinity than hemoglobin A and a much higher oxygen affinity than hemoglobin S being able to bind 2,3 bi-phosphoglycerate less strongly. In the HbF γ chain at birth, γ^G is more abundant, while γ^A is the predominant form in adulthood.[12] The α gene remains fully active at birth, following delivery, the γ is downregulated while more of the β and less of δ genes are up-regulated. Combinations of α gene with β,δ and γ genes give rise to the formation of 97% normal adult hemoglobin A (α_2 β_2), 2% hemoglobin A_2 (α_2 δ_2) and 1% of hemoglobin F (α_2 γ_2) by the end of the first year of life. In individuals whose γ gene downregulation is not effective following delivery, it results in higher percentage of HbF (α_2 γ_2) known as hereditary persistent of fetal hemoglobin HPFH.[13] When glucose is covalently glycosylated to the β-chain amino-terminal residue it forms hemoglobin A_{1C}.[14]

Fetal hemoglobin and its benefits in sickle cell anemia

Xu *et al*. in their study reported amelioration of the symptoms associated with SCA in individuals with accentuated expression of γ globin genes resulting in a high level of HbF which compensates for the defective β-globin products of SCA.[15] High HbF levels are inherited as quantitative traits dependent on many gene loci outside the β-globin cluster[16] including 2q16, 6q23, 8q, and Xp22.2.[17] As early as 1948, Janet Watson noted that symptoms associated with SCA were not fully manifested until a hemoglobin switch from fetal to adult takes place.[18] Several laboratory studies have confirmed levels of HbF needed to ameliorate symptoms associated with SCA and high level of HbF is reported to be inversely related to degree of severity in SCA.[19]

High HbF retards polymerization of sickled cells in the deoxygenated state by reducing HbS concentration thus inducing a lower rate of vaso-occlusive crises, leg ulcers, avascular necrosis of the neck of femur, acute chest syndrome, and ultimately a reduced disease severity. However, elevated HbF does not impact on priapism, stroke, systemic blood pressure, and sickle vasculopathy.[20]

The percentage of F cells in females is reported to be higher than in males because it is partially controlled by the Xp 22.2 locus on the X chromosome. A total of 70% of the variation in HbF levels in SCA patients may be associated with variation in percentages of F cells.[21]

Olaniyi *et al*.[22] in 2010 determined the HbF ranges in Nigerian SCA patients, they categorized the level into low HbF <2% moderate 2.1%–10% and high 10.1%–16%.[22]

Hemoglobin A_2 and implications of its high level in sickle cell anemia

When compared with HbSS individuals, HbA_2 has been reported to be elevated in Hb S/β^0-thalassemia.[23] An advantage of quantification of HbA_2 level is to differentiate between the two types of sickle cell anemia, i.e. Hb SS and Hb S/β^0-thalassemia.[24,25]

High-performance liquid chromatography (HPLC) is regarded as the method of choice by many researchers for the measurement of HbA_2 in patients with SCA.[26-28] However, this was faulted by Suh *et al*. in 1996.[29]

They proposed that HbA_2 estimation in SCA may be falsely estimated by HPLC because HbA_2 values obtained by HPLC increased significantly in samples containing HbS resulting in wrongly labeling HbSS as Hb S/β^0-thalassemia.[29] Based on 1996 Suh *et al.* hypothesis, HPLC analyzed HbA_2 value of up 5.9% in HbSS individuals was considered as normal value by Shokrani *et al*. in 2000.[30]

Posttranslational modifications in some HbS make them have same retention time as HbA_2. This was postulated as the reason for falsely elevated HPLC measured HbA_2 in patients with SCA by Head *et al*. in 2004.[24] The presence of other β chain variants such as in HbE, HbD, and Hb Lepore has also impacted on the falsely elevated levels of HbA_2 measured by HPLC.[31,32]

However, Giambona *et al*.[33] defined borderline β-thalassemia as HbA_2 of a range of between 3.1%-3.9%, in an Italian population with a high prevalence of β-thalassemia, in which a total of 23,485 patients were retrospectively studied between 2000 and 2006.

Materials and Methods

Study population

Patients attending adult SCA Clinics of Lagos State University Teaching Hospital, Ikeja, were the study population. The clinic has an estimated total of 500 patients with SCA.

Inclusion criterion

HPLC Hemoglobin quantification results of homozygous HbSS patients found in the case notes.

Exclusion criteria

1. HPLC Hemoglobin quantification results of HbSC or other double heterozygous phenotype such as HbSD, and Hb CD, etc
2. All HbSA patients
3. All patients already on hydroxyurea

4. HIV-positive HbSS patients
5. HbSS participants with elevated mean corpuscular volume >100.

Study design

This was a descriptive, cross-sectional, and retrospective study involving SCD patients. The study was conducted in November 2017. Patients' sociodemographic data and the HPLC results were retrieved from their folders. Proportions of HbA_2, HbF, and HbS of the patients were generated. HbF values were categorized as done by Olaniyi *et al*. and HbA_2 by Giambona *et al*.

Sampling technique

A nonprobability convenience sampling was used that is applicable to both quantitative and qualitative studies, in which members of a target population that meet certain criteria, such as accessibility, geographical proximity, availability at a given time, or willingness to participate are included for the purpose of the study. Percentages of HbA_2, HbF, and HbS from the case notes were abstracted.

Data analysis

The data were entered and analyzed using IBM SPSS Statistics for Windows, Version 20.0 Armonk, New York, USA. The mean ± standard deviation (SD), median, SDs were generated as necessary for continuous data. Tests of statistical significance between variables such as Chi-square analysis and Fischer's exact for discrete data were used. Level of significance was set at $P \leq 0.05$.

Ethical consideration

Ethical approval was obtained from Health and Ethics Research Committee. The reference number is LREC.06/10/916, approved on October 10, 2017.

Results

One hundred (100) out of a total of 129 participants who met the inclusion and exclusions criteria were used. It consisted of 40 (40%) males and 60 (60%) females. The overall mean ± SD age of participants was 25.89 ± 9.34 years. The overall mean ± SD HbF was 6.94%±5.05. Table 1 shows overall and gender-specific parameters of age, HbF, HbA_2, and HbS.

The overall mean ± SD HbA_2 was 3.75%±0.74. Table 2 shows overall and gender-specific HbF ranges. Analyzing HbA_2 in all participants, Table 3 highlights overall and gender-specific Hb A_2 ranges.

The mean age of male participants was 23.77 ± 8.13 years with a range of 14 and 49 years [Table 1]. The mean HbF for males was 6.97 ± 5.45%, a minimum of 1% and maximum of 21.6%. HbF values for males showed majority 24 (60%) had a range of between 2.1% and 10%, followed by 10.1% and 16% and <2%, both groups had 6 of 40 each (15%) and only 4 (10%) had HbF >16.1% [Table 2].

Majority of males participants 25 (62.5%) had HbA_2 of 3.1%–3.9%, followed by 10 (25%) with a range of 4%–5.9% and 5 (12.5%) had $HbA_2 < 3.1$ [Table 3].

Table 1: Overall and gender-specific mean age, hemoglobin F, hemoglobin A_2 and hemoglobin S

Parameters	Males ($n=40$)	Females ($n=60$)	Total ($n=100$)
Age (years)	23.77±8.13	27.29±9.88	25.89±9.34
HbF (%)	6.97±5.45	6.92±4.87	6.94±5.08
HbA_2 (%)	3.68±0.58	3.80±0.83	3.75±0.74
HbS (%)	87.34±7.06	86.34±5.76	86.74±6.3

HbF: Haemoglobin F, HbA_2: Haemoglobin A_2, HbS: Haemoglobin S

Table 2: Overall and gender-specific hemoglobin F ranges

HbF levels (%)	Males (%)	Females (%)	Total (%)
<2	15	15	15
2.1-10	60	63.3	62
10.1-16	15	18.3	17
>16	10	3.4	6

HbF: Haemoglobin F

Table 3: Overall and gender-specific hemoglobin A_2 ranges

HbA_2 levels (%)	Males (%)	Females (%)	Total (%)
<3.1	12.5	18.3	16
3.1-3.9	62.5	41.7	50
4-5.9	25	40	34
>6	0	0	0

HbA_2: Hemoglobin A_2

The mean HbF for females was 6.92 ± 4.87%. HbF values for females showed, majority 38 (63.3%) had HbF of between 2.1% and 10% followed by 11 (18.3%) with HbF of between 10.1% and 16%, similarly, 9 (15%) had HbF of <2% and only 2 (3.3%) females had HbF >16.1% [Table 2]. The mean HbA_2 was 3.80 ± 0.83. Majority 25 (41.7%) are within the range of 3.1%–3.9%, followed by 24 (40%) with a range of 4%–5.9% and 11 (18.3%) had a range of <3.1% [Table 3].

HbF and HbA_2 *P* values were 0.56 and 0.44, respectively, when compared with the ages of participants, similarly, HbF and HbA_2 *P* values were 0.53 and 0.43, respectively, when compared with the gender of participants.

Discussions

HPLC has been proven to be a rapid, sensitive and accurate method for quantifying various types of normal and abnormal hemoglobins despite its limitations, one of which includes co-elution of HbS byproducts with HbA_2 resulting in falsely elevated level of the latter.[34] It is however very suitable for HbF estimation with little or no limitations.

This study analyzed HPLC generated results of HbF and HbA_2 in one hundred SCA patients with a view to determining their, overall and gender-specific reference values for HbF and HbA_2 in Lagos, South West, Nigeria, determine the percentage of our SCA patients with low, moderate or high values of HbF, the value has been proven to impact on the clinical severity

of SCA patients and to determine SCA patients with elevated HbA_2 which is a reliable diagnostic marker of beta thalassemia trait.

Epidemiological study has demonstrated that the lower rates of recurrent clinical events such as acute chest syndrome, vaso-occlusive crises, and frequent rate of hospitalizations are associated with HbF levels above 20%.[35] Similarly, patients with values above 10% had reduced incidence of strokes and avascular necrosis of head of femur.[35]

This study reported a mean HbF value of 6.94% ±5.05, which is lower than the previous studies done in, Lagos,[36] but similar to values reported in Ibadan,[37] and Benin.[38]

Despite the wide range noted in this study, we observed that 7% of SCA patients in this study have HbF levels below 1% unexpectedly much lower than 14% reported in HbAA individuals, although, methodology used differs,[39] 20% had HbF ≥10% which confers advantage of reduced incidence of strokes and avascular necrosis of the head of femur.[35] However, majority of the participants in this study (62%) had value between 2% and 10%. Only 2% have HbF >20% which is considered as hereditary persistent of fetal hemoglobin (HPFH) coexisting with HbS.[14] HPFH is caused by deletions of the β-globin gene cluster, which induces a compensatory γ-globin synthesis increase. It is also thought to be due to mutations in the HBG promoter regions or inheritance of HbF modulating quantitative trait loci, like HBSIL-MYB intergenic region (6q23) and BCL11A (2p16).[40,41]

In a contrary study by Adeyemo *et al*.,[36] they reported a higher mean value in females than males as compared to this study. The study in Ibadan reported a higher value in males than females despite a reported higher percentage of F cells in females than males and HbF levels being partially controlled by an X-Linked Gene.[21] However, the value obtained in this study is not statistically significant when compared to the study by Adeyemo *et al*.[36]

Another point worthy of note is 7%, and 23% of our patients had very low HbF <1% and low level of HbF <3%, respectively. These groups might benefit from hydroxycarbamide, which is known to elevate HbF level by conversion of the hydroxycarbamide to nitric oxide (NO) *in vivo*. NO stimulates intracellular soluble guanylate cyclase which in turn, elevates cGMP and causes an increase of HbF level through cGMP-dependent protein kinase G.[42]

SCA patients with variants in BCL11A have been reported to have a higher HbF response to hydroxycarbamide[43] elevated HbF improves or reduces severity of crises in the patients.[44] However, Green *et al*. in 2016 reported a blunted response of HbF to hydroxycarbamide use overtime leaving SCA patients to worsening disease complications and increased hospitalizations.[45]

Determining HbA_2 levels in SCA in order to identify SCA patients with beta thalassemia trait may not have physiological significance in SCA, its knowledge is desirable in genetic counseling for couples at risk of having affected child with β-thalassemia[46] Apart from using elevated HbA_2 to diagnose beta thalassemia trait, which is considered the best approach for beta thalassemia trait diagnosis, various methods could help in making in the diagnosis of beta thalassemia trait, these include microcytic (M)/hypochromic (H) ratio estimation,[47] red blood cell flags,[48] red cell distribution width.[49] Adeyemo *et al*. in 2014 proposed that elevated HbA_2 in SCA patients above 4%[36] is a valuable screening tool in diagnosing beta-thalassemia trait, while borderline range of beta thalassemia trait is considered to be 3.1%–3.9% of HbA_2.[24,33,50]

However, apart from SCA, other factors that could cause elevation of HbA_2 above 3.4% are thyrotoxicosis, HIV infection with zidovudine therapy and some cases of megaloblastic anemia. Furthermore, alpha thalassemia, severe iron deficiency, anemia of chronic diseases, sideroblastic anemia, lead poisoning, and acute myeloid leukemia could cause a reduction on HbA_2 lower than 2.2%.[51,52]

The mean HbA_2 obtained in this study was very similar to value obtained from SCA patients in Brazil in which the authors quantified HbA_2 with HPLC.[53]

Another study by Craver *et al*.[54] reported a lower value of HbA_2 as compared to this study. This could be due to the fact that isoelectric focusing was used by Craver for the quantification of HbA_2 underscoring substantial co-elution of HbS with A_2 byproducts in HPLC resulting in falsely elevated level. However, the three values in SCA are much higher than HbAA reference range (2.4% ±0.9) reported by Craver *et al*.[54]

Thirty-four percent of our study participants had HbA_2 > 4% hence could be considered to be SCA with beta thalassemia trait and wrongly labeled as HbSS. This is higher than the proportion of participants with SCA with beta-thalassemia trait reported by Adeyemo *et al*. in their study probably because unlike this study they considered red cell indices as a screening tool apart from level of HbA_2 > 4%.[36] Our reported value is also higher than value reported by Inusa *et al*. in Northern Nigeria in 2015 because they used a higher HbA_2 cutoff value of 6% and the general population unlike SCA cohort used in this study.[55]

Thalassemia is considered a Mediterranean disease, the presence of beta thalassemia trait among our sickle cell population in Lagos, Nigeria which is a non-Mediterranean country might be higher than excepted going by this study, a polymerase chain reaction (PCR)-based method to determine the exact percentage of the beta thalassemia in our population is the gold standard and long overdue.

Conclusions

Thirty percent of the study participants had HbF <3% while 34% of them had elevated HbA_2 level >4% and could indeed be carrying beta thalassemia trait with the sickle cell gene.

Limitations

A limitation of the study is reliability on the HPLC measured HbA_2 percentages despite its widely reported deficiencies. Another important limitation of this study is the use of hospital-based data to determine reference range instead of data generated from a population-based study, lack of use of controls and appropriate matching, done in a community survey. A more sensitive test like PCR-based study will be appropriate to confirm a diagnosis of thalassemia in the absence of a population-based study.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. World Health Assembly: Resolutions and Decisions Annexes. WHA 59/2006/REC/1. Geneva: World Health Organization; 2006.
2. Ingram VM. A specific chemical difference between the globins of normal human and sickle-cell anemia haemoglobin.Nature.1956;178:792-794.
3. Embury SH, Dozy AM, Miller J, Davis JR Jr., Kleman KM, Preisler H, *et al.* Concurrent sickle-cell anemia and alpha-thalassemia: Effect on severity of anemia. N Engl J Med 1982;306:270-4.
4. Higgs DR, Aldridge BE, Lamb J, Clegg JB, Weatherall DJ, Hayes RJ, *et al.* The interaction of alpha-thalassemia and homozygous sickle-cell disease. N Engl J Med 1982;306:1441-6.
5. Murray CJ, Ezzati M, Flaxman AD, Lim S, Lozano R, Michaud C, *et al.* GBD 2010: A multi-investigator collaboration for global comparative descriptive epidemiology. Lancet 2012;380:2055-8.
6. Fleming AF, Storey J, Molineaux L, Iroko EA, Attai ED. Abnormal haemoglobins in the sudan savanna of Nigeria. I. Prevalence of haemoglobins and relationships between sickle cell trait, malaria and survival. Ann Trop Med Parasitol 1979;73:161-72.
7. Galadanci N, Wudil BJ, Balogun TM, Ogunrinde GO, Akinsulie A, Hasan-Hanga F, *et al.* Current sickle cell disease management practices in Nigeria. Int Health 2014;6:23-8.
8. Akinyanju OO. A profile of sickle cell disease in Nigeria. Ann N Y Acad Sci 1989;565:126-36.
9. Perutz MF. X-Ray Analysis of Haemoglobin. Stockholm: Les Prix Nobel; 1963. Science is not a Quiet Life: Unraveling the Atomic Mechanism of Haemoglobin. London: Imperial College Press; 1997.
10. Antonini E, Brunori M. Hemoglobin and Myoglobin in Their Reactions with Ligands. Amsterdam: North-Holland:North-Holland; 1971.
11. Schechter AN. Hemoglobin research and the origins of molecular medicine. Blood 2008;112:3927-38.
12. Zago MA, Falcão RP, Pasquini R. Hematologia: Fundamentos e Prática. Ed Rev Atual. São Paulo: Editora Ateneu; 2004. p. 245.
13. Forget BG. Molecular basis of hereditary persistence of fetal hemoglobin. Ann N Y Acad Sci 1998;850:38-44.
14. Bunn HF, Gabbay KH, Gallop PM. The glycosylation of hemoglobin: Relevance to diabetes mellitus. Science 1978;200:21-7.
15. Xu XS, Hong X, Wang G. Induction of endogenous gamma-globin gene expression with decoy oligonucleotide targeting oct-1 transcription factor consensus sequence. J Hematol Oncol 2009;2:15.
16. Thein SL, Menzel S. Discovering the genetics underlying foetal haemoglobin production in adults. Br J Haematol 2009;145:455-67.
17. Nguyen TK, Joly P, Bardel C, Moulsma M, Bonello-Palot N, Francina A, *et al.* The xmnI (G)gamma polymorphism influences hemoglobin F synthesis contrary to BCL11A and HBS1L-MYB SNPs in a cohort of 57 beta-thalassemia intermedia patients. Blood Cells Mol Dis 2010;45:124-7.
18. Watson J. The significance of the paucity of sickle cells in newborn negro infants. Am J Med Sci 1948;215:419-23.
19. Noguchi CT, Rodgers GP, Serjeant G, Schechter AN. Levels of fetal hemoglobin necessary for treatment of sickle cell disease. N Engl J Med 1988;318:96-9.
20. Steinberg MH, Forget BG, Higgs DR, Weatherall DJ. Disorders of Haemoglobin: Genetics, Pathophysiology, Clinical Management. 2nd ed. Cambridge,United Kingdom: Cambridge University Press; 2009.
21. Dover GJ, Smith KD, Chang YC, Purvis S, Mays A, Meyers DA, *et al.* Fetal hemoglobin levels in sickle cell disease and normal individuals are partially controlled by an X-linked gene located at xp22.2. Blood 1992;80:816-24.
22. Olaniyi JA, Arinola OG, Odetunde AB. Foetal haemoglobin (Hbf) status in adult sickle cell anaemia patients in Ibadan, Nigeria. Ann Ib Postgrad Med 2010;8:30-3.
23. Sweeting I, Serjeant BE, Thomas PW, Serjeant GR. Microchromatographic quantitation of Hb A2 levels in phenotypes of sickle cell-beta(+) thalassemia. J Chromatog B 1997;700:269-274.
24. Head CE, Conroy M, Jarvis M, Phelan L, Bain BJ. Some observations on the measurement of haemoglobin A2 and S percentages by high performance liquid chromatography in the presence and absence of alpha thalassaemia. J Clin Pathol 2004;57:276-80.
25. Clarke GM, Higgins TN. Laboratory investigation of hemoglobinopathies and thalassemias: Review and update. Clin Chem 2000;46:1284-90.
26. Greene DN, Vaughn CP, Crews BO, Agarwal AM. Advances in detection of hemoglobinopathies. Clin Chim Acta 2015;439:50-7.
27. Greene DN, Pyle AL, Chang JS, Hoke C, Lorey T. Comparison of sebia capillarys flex capillary electrophoresis with the bioRad variant II high pressure liquid chromatography in the evaluation of hemoglobinopathies. Clin Chim Acta 2012;413:1232-8.
28. Paleari R, Gulbis B, Cotton F, Mosca A. Interlaboratory comparison of current high-performance methods for hbA2. Int J Lab Hematol 2012;34:362-8.
29. Suh DD, Krauss JS, Bures K. Influence of hemoglobin S adducts on hemoglobin A2 quantification by HPLC. Clin Chem 1996;42:1113-4.
30. Shokrani M, Terrell F, Turner EA, Aguinaga MD. Chromatographic measurements of hemoglobin A2 in blood samples that contain sickle hemoglobin. Ann Clin Lab Sci 2000;30:191-4.
31. Higgins TN, Khajuria A, Mack M. Quantification of hbA(2) in patients with and without beta-thalassemia and in the presence of hbS, hbC, hbE, and hbD Punjab hemoglobin variants: Comparison of two systems. Am J Clin Pathol 2009;131:357-62.
32. Mosca A, Paleari R, Ivaldi G, Galanello R, Giordano PC. The role of haemoglobin A(2) testing in the diagnosis of thalassaemias and related haemoglobinopathies. J Clin Pathol 2009;62:13-7.
33. Giambona A, Passarello C, Vinciguerra M, Li Muli R, Teresi P, Anzà M, *et al.* Significance of borderline hemoglobin A2 values in an Italian population with a high prevalence of beta-thalassemia. Haematologica 2008;93:1380-4.
34. Bio Rad. Variant Beta-Thallasemia Short Program Procedure Manual. Hercules,CA: Bio Rad Laboratories Inc; 1994.
35. Powars DR, Weiss JN, Chan LS, Schroeder WA. Is there a threshold level of fetal hemoglobin that ameliorates morbidity in sickle cell anemia? Blood 1984;63:921-6.
36. Adeyemo T, Ojewunmi O, Oyetunji A. Evaluation of high performance liquid chromatography (HPLC) pattern and prevalence of beta-thalassaemia trait among sickle cell disease patients in Lagos, Nigeria. Pan Afr Med J 2014;18:71.
37. Kotila TR, Fawole OI, Shokunbi WA. Haemoglobin F and clinical severity of sickle cell anaemia among Nigerian adults. Afr J Med Med Sci 2000;29:229-31.
38. Enosolease ME, Ejele OA, Awodu OA. The influence of foetal haemoglobin on the frequency of vaso-occlusive crisis in sickle cell anaemia patients. Niger Postgrad Med J 2005;12:102-5.
39. Kotila TR, Shokunbi WA. Haemoglobin F levels in healthy Nigerian adults. West Afr J Med 2003;22:143-5.
40. Akinsheye I, Alsultan A, Solovieff N, Ngo D, Baldwin CT, Sebastiani P, *et al.* Fetal hemoglobin in sickle cell anemia. Blood 2011;118:19-27.
41. Galarneau G, Palmer CD, Sankaran VG, Orkin SH, Hirschhorn JN, Lettre G, *et al.* Fine-mapping at three loci known to affect fetal hemoglobin levels explains additional genetic variation. Nat Genet 2010; 42:1049-51.

42. Almeida CB, Scheiermann C, Jang JE, Prophete C, Costa FF, Conran N, *et al.* Hydroxyurea and a cGMP-amplifying agent have immediate benefits on acute vaso-occlusive events in sickle cell disease mice. Blood 2012;120:2879-88.
43. Friedrisch JR, Sheehan V, Flanagan JM, Baldan A, Summarell CC, Bittar CM, *et al.* The role of BCL11A and HMIP-2 polymorphisms on endogenous and hydroxyurea induced levels of fetal hemoglobin in sickle cell anemia patients from Southern Brazil. Blood Cells Mol Dis 2016;62:32-7.
44. Platt OS, Orkin SH, Dover G, Beardsley GP, Miller B, Nathan DG, *et al.* Hydroxyurea enhances fetal hemoglobin production in sickle cell anemia. J Clin Invest 1984;74:652-6.
45. Green NS, Manwani D, Qureshi M, Ireland K, Sinha A, Smaldone AM. Decreased fetal haemoglobin overtime among you with sickle cell disease on hydroxyurea is associated with higher urgent hospital use. Pediatr Blood Cancer 2016;63;2146-53.
46. Giambona A, Passarello C, Renda D, Maggio A. The significance of the hemoglobin A(2) value in screening for hemoglobinopathies. Clin Biochem 2009;42:1786-96.
47. Ntaios G, Chatzinkolaou A. M/H ratio for the differential diagnosis of macrocytic anaemia. Int J Lab Hematol 2009:31:248-9.
48. Eivazi-Ziaei J, Dastgiri S, Pourebrahim S, Soltanpour R. Usefulness of red blood cell flags in diagnosing and differentiating thalassemia trait from iron-deficiency anemia. Hematology 2008;13:253-6.
49. Harrington AM, Ward PC, Kroft SH. Iron deficiency anemia, beta-thalassemia minor, and anemia of chronic disease: A morphologic reappraisal. Am J Clin Pathol 2008;129:466-71.
50. Steinberg MH, Adams JG 3rd. Hemoglobin A2: Origin, evolution, and aftermath. Blood 1991;78:2165-77.
51. Figueiredo MS. The importance of hemoglobin A2 determination. Rev Bras Hematol Hemoter 2015;37:287-9.
52. Bain BJ, Wild BJ, Stephens AD, Bhalan LA. Variant Haemoglobins, A Guide to Identification. 1st ed. West Sussex.UK: Wiley-Blackwell; 2010.
53. da Fonseca SF, Amorim T, Purificação A, Gonçalves M, Boa-Sorte N. Hemoglobin A2 values in sickle cell disease patients quantified by high performance liquid chromatography and the influence of alpha thalassemia. Rev Bras Hematol Hemoter 2015;37:296-301.
54. Craver RD, Abermanis JG, Warrier RP, Ode DL, Hempe JM. Hemoglobin A2 levels in healthy persons, sickle cell disease, sickle cell trait, and beta-thalassemia by capillary isoelectric focusing. Am J Clin Pathol 1997;107:88-91.
55. Inusa BP, Daniel Y, Lawson JO, Dada J, Mathews CE, Momi S. Sickle cell screening in Nothern Nigeria. Coexistence β-Thalassaemia Herit Pediat Ther 2015;5;262.