The Percentage Levels of HbS, HbF and HbA₂ in Patients with Sickle Cell Diseases Using HPLC: A Diagnostic Guide to the Physicians

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Abstract

Background Sickle cell disease is one of the common genetic diseases worldwide and it is of high prevalence in Middle-east, Mediterranean region, Southeast Asia and Sub-Saharan Africa. The aim of this study was to determine the percentage levels of HbS, HbA₂ and HbF in steady state sickle cell disease patients in order to guide the physicians in the diagnosis and management of sickle cell disease patients. Methodology A total of one-hundred and forty-eight (148) sickle cell disease patients (128HbSS- and 20 Hb SC- patients), aged 1 to 33 years, and thirty, aged-matched, apparently healthy subjects with Hb AA (control group) were recruited for the study between January 2014 and February 2016. Hemoglobin levels of S, A₂ and F were determined by ion-exchange HPLC. Results The mean hemoglobin levels for S, A₂ and F were 81.1±4.98%, 2.9±0.81% and 7.2± 4.1 %, respectively in steady state sickle cell anemia patients. The mean values of HbS, HbA₂ and HbF of 82.18±5.3%, 2.78 ± 0.79% and 6.37 ± 4.09% in males compared to 80.5 ± 4.73%, 2.97 ± 0.82% and 7.7 ± 4.05%, respectively in females showed no statistically significant differences (P>0.05). However, the mean HbF levels of 0.56 ± 0.56%, 7.2 ± 4.1% and 2.34 ± 2.54% for Hb AA, Hb SS and Hb SC patients, respectively showed statistically significant difference (P<0.05) while mean HbA₂ levels of 2.96 ± 0.81%, 2.9 ± 0.81% and 3.4 ± 1.27% for Hb AA, Hb SS and Hb SC patients, respectively showed no statistically significant difference (P>0.05). Conclusion: Significantly higher level of HbF was observed in steady state SCD patients irrespective of gender while HbA₂ levels in Hb AA, Hb SS and Hb SC patients showed no significant difference irrespective of gender. However, it is recommended that the established hemoglobin levels of S, A₂ and F will serve as guide to the physicians in the diagnosis and management of SCD patients.

Keywords: Hemoglobin percentage, steady state, sickle cell disease, Nigeria.

Introduction

Sickle cell disease (SCD) is of the most common genetic diseases worldwide and its high prevalence has been reported in Middle east, Mediterranean region, Southeast Asia and Sub-Saharan Africa and most especially in Nigeria. [1,2] Sickle cell diseases consist of a group of disorders characterized by the presence of sickle hemoglobin. Structural hemoglobin variants identified are over 700 but only two (HbS and HbC) have reached high frequencies in Africa. [3] The common SCD syndromes in this region include homozygous Hb SS disease, commonly known as sickle cell anemia (SCA) and Hb SC disease. [3] The sickle hemoglobin (HbS) mutation occurred independently at least four times in region with endemic malaria. In the heterozygous state, the sickle mutation provides protection against infection by the falciparum species of malaria and likely confers a survival advantage, leading to it is continue high prevalence in some population of Sub-Saharan Africa, Middle-east and India. [4] Many patients with SCA are in reasonably good health, most of the time and they achieve a steady state level of fitness. However, the state of relative well-being can be periodically interrupted by crisis such as
the vaso-occlusive crisis (VOC), which is the most common. [5] A patient with SCA is said to be in steady state when there is absence of infection, acute complicating factors or acute clinical symptoms or crisis for at least three months. [6] Inter-individual variation in foetal haemoglobin (HbF) levels is one of the main modifiers that contribute to clinical heterogeneity observed in patients. Higher expression of HbF in adulthood ameliorates morbidity and mortality in SCD, [7, 8] as increased level of HbF has been observed to have beneficial effect in sickle cell anaemia due to the inhibition of polymerization of HbS which results in erythrocyte sickling. [9,10] Hemoglobin A2 forms less than 3% of total hemoglobin in normal adults [11] and it has a pan cellular distribution, and ability to inhibit the polymerization of sickle hemoglobin (HbS) [12, 15].

Increased expression of HbA2 has been further linked to the compensation for the impaired β-globin production inherent in β-haemoglobinopathies and therefore ameliorates the clinical severity of these diseases. [16] High level of HbS of about 80-90% seen in homozygous disease is associated with a worse disease while the presence of alpha thalassemia (one or two gene deletions) ameliorates the disease. [17] However, many studies recommended decreasing HbS level in these patients to<30% in order to reduce the risks associated with stroke, recurrent infarction and perioperative complications [18-21].

Exchange or red cell transfusions are parts of the procedures that do not only ameliorate the symptoms of anaemia by increasing the haematocrit in patients with sickle cell anaemia but also lower the fraction of HbS in the circulation and thereby decrease the risk of end-organ damage in these patients. [18-24] Reports on the levels of HbA2, HbF and HbS among patients with sickle cell disease vary widely in different parts of the world. In Nigeria, the range of 3.76 - 4.53% for HbA2 has been documented [25, 26] while other countries reported a range of 2.67-4.09%. [27-30].

However, the documented values of HbF in patients with SCA were between 2.17 and 8.05% in Nigeria [25, 26, 31] while others had a range of 6.8-21.3% of HbF. [27,28 ,30 ,32] The level of hemoglobin S in patients with sickle cell anaemia was of a range of 77.7 -

86.7%. [26, 28, 33] In the past, the procedures available for the diagnosis of haemoglobinopa this in Nigeria were limited to a few screening tests such as the sickling and solubility tests as well as other qualitative tests such as cellulose acetate electrophoresis.

However, few centers in Nigeria have introduced the use of high performance liquid chromatography (HPLC) technology which is capable of identifying and quantifying hemoglobin variants. HPLC improves the accuracy of diagnosis and eases the monitoring of therapies that are associated with hydroxyurea and exchange blood transfusion (EBT) among other benefits. Therefore, as a result of the increasing availability of the HPLC in Nigeria, the study was aimed to determine the levels of Hbs, HbA2 and HbF in patients with sickle cell disease who were in steady state in order to serve as guide in the diagnosis and monitoring of therapies in these patients.

Materials and Methods

A total of one-hundred and forty-eight (148) sickle cell disease patients in steady state, that were made up of 128 HbSS patients (83 females and 45 males) and 20 HbSS patients (13 females and 7 males), and aged 1-33 years, were recruited for the study from the Sickle Cell Clinic of Aminu Kano Teaching Hospital, Kano between January 2014 and February 2016 while thirty (15 males and 15 females), age-matched, apparently healthy individuals (HbAA) were used as controls.

Inclusion Criteria

Patients with Hb SS and Hb SC who gave their consent and diagnosed by cellulose acetate electrophoresis at pH 8.6, and was in steady state. Steady state in SCD was defined based on a patient having stable haematocrit and hemoglobin values over a period of 2-3 clinic visits at 4-6 weeks interval, a state of well-being without any symptoms or signs suggestive of crisis established by a careful history and complete physical examination for the past 3 months and there was no history of blood transfusion for the past 3 months. [34, 35]

Exclusion Criteria

Sickle cell disease patients who gave no consent or had blood transfusion within the...
past 3 months or crisis was excluded from the study.

**Ethical Approval and Sample Collection**

After the ethical approval from the ethical committee of Aminu Kano Teaching Hospital, Kano and informed consent sought from the patients and parents or guardians, 2ml of venous blood sample was collected from each patient or control subject into ethylene diamine tetra-acetic acid bottle and mixed gently and thoroughly. Hemoglobin levels of S, A2 and F were estimated on each blood sample according to the manufacturer’s instruction of Bio-Rad D-10 dual program on ion-exchange HPLC (Bio-Rad Laboratories, Inc., Hercules USA). [36]

**Data Analysis**

Data were analyzed using the statistical package for the social sciences (SPSS) version 19, inc., Chicago, ILL and the descriptive data were given as mean ± standard deviation. Student t- test and analysis of variance were employed to assess the significance of the differences among two and three groups, respectively. The differences were considered to be statistically significant with p-values < 0.05.

**Results**

Table 1 shows hemoglobin levels of S, A2 and F in steady state SCA patients. The mean values of HbS, HbA2 and HbF were 81.1 ± 4.98%, 2.9 ± 0.81 and 7.2 ± 4.1%, respectively. The hemoglobin levels of S, A2 and F in steady state SCA patients according to gender are shown in table 2. There were no statistically significant differences in the values of HbS, HbA2 and HbF of 82.18 ± 5.3%, 2.78 ± 0.79% and 6.37 ± 4.09% in males compared to 80.5 ± 4.73%, 2.97 ± 0.82% and 7.7 ± 4.05% in females, respectively (P>0.05).

Table 3 shows comparison of hemoglobin levels of A2 and F in steady state sickle cell disease patients with controls (HbAA subjects). There was no statistically significant difference in the hemoglobin levels of A2 of 2.96 ± 0.81%, 2.9 ± 0.81% and 3.4 ± 1.27% for HbAA, HbSS and HbSC subjects or patients, respectively (P>0.05) while statistically significant difference was observed in foetal hemoglobin (HbF) levels of 0.56 ± 0.56%, 7.2 ± 4.1% and 2.34 ± 2.54% for HbAA, HbSS and HbSC subjects or patients, respectively (P<0.05)

Comparison of HbA2 and HbF levels in steady state SCA patients with controls (Hb AA subjects) according to gender is revealed in table 4. The mean values of HbA2 for male control subjects (Hb AA) and male Hb SS patients were 3.0 ± 0.95% and 2.8 ± 0.79% respectively while the mean values of HbA2 for female control subjects (Hb AA) and female Hb SS patients were 2.92 ± 0.67% and 2.97 ± 0.82%, respectively. There were no statistically significant differences observed with regard to HbA2 levels in males and females (P>0.05).

The statistical analysis showed significantly higher levels of HbF in males with Hb SS (6.37 ± 4.09%) compared to control group of Hb AA (0.45 ± 0.44%), and in females with Hb SS (7.7 ± 4.05%) compared to Hb AA control group (0.67 ± 0.66%(P<0.05).

| Table 1: Hemoglobin levels of S, A2 and F in steady state SCA patients |
|------------------|------------------|------------------|
|                   | HbS              | HbA2             | HbF              |
| Number            | 128              | 128              | 128              |
| Level (%)         | 81.1±4.98        | 2.9±0.81         | 7.2±4.1          |

| Table 2: Hemoglobin levels of S, A2 and F in steady state SCA patients according to gender |
|-----------------------------------------------|------------------|------------------|
|                   | Hb S (%)         | Hb A2 (%)        | Hb F (%)         |
| Males (n=45)      | 82.81±5.3        | 2.78±0.79        | 6.37±4.09        |
| Females (n=83)    | 80.5±4.73        | 2.97±0.82        | 7.7±4.05         |
| P-value           | 0.0684           | 0.2073           | 0.0795           |

| Table 3: Comparison of hemoglobin levels of A2 and F in steady state SCD patients and control group (Hb AA) |
|-----------------------------------------------|------------------|------------------|------------------|
|                   | HB AA (CONTROL GROUP) | HB SS PATIENTS | HB SC PATIENTS | P-VALUE |
| NUMBER            | 30                | 128             | 20              |
| Hb A2 (%)         | 2.96±0.81         | 2.9±0.81        | 3.4±1.27        | 0.0608  |
| Hb F (%)          | 0.56±0.56         | 7.2±4.1**       | 2.34±2.54*      | 0.000   |

*Hb F level in HbSS compared to control group (HbAA) (P=0.0001)
*Hb F level in HbSC compared to control group (HbAA) (P=0.0005)
Table 4: Comparison of HbA2 and HbF levels in steady state SCA patients and HbAA (controls) according to gender

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<th>p-value</th>
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<td>Hb A2 (%)</td>
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<td>Number</td>
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<td>Hb F (%)</td>
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Discussion

The importance of the determination of hemoglobin levels of S, A2 and F in sickle cell disease patients for better management of patients cannot be overemphasized, hence their quantitation using ion-exchange HPLC.

The study has shown the mean value of 81.1% for HbS in steady state sickle cell disease patients and this agrees with the earlier reports, [26, 28, 33] however, recommendation for the reduction in Hb S level to less than 30% in SCD patients has been emphasized by various studies to reduce the complications such as stroke and recurrent acute chest syndrome that are associated with the disease. [18 – 24]

The mean value of 2.9% for HbA2 obtained among the SCA patients in this study agrees with the range of 2.7 - 4.1% for HbA2 reported by various authors. [27-30] However, our finding is less than the range of 3.76 - 4.52% of HbA2 reported in Southern Nigeria. [25, 26] The mild variation in HbA2 levels from different studies could be associated with specificities of the techniques employed, sample numbers, and probably the lumping of patients with sickle cell anemia together irrespective of their status. However, high HbA2 level has been associated with inhibition of the polymerization of HbS in experimental models. [16]

The study has further revealed the mean value of HbF in steady state sickle cell anemia patients of 7.2% which supports the previous reports of 2.2 – 8.1% documented in Nigeria [25, 26, 31] but in contrary with the significantly higher values of HbF of 12.8-21.5 observed in other studies associated with Arabs countries. [27, 32, 33] This striking increase in HbF values amongst SCA patients in the Arab countries may be genetic. However, the significantly high HbF levels in SCD patients in some of the Arab countries have been associated with Saudi Arabia/ Indian (SAI) or the Senegal haplotype of the HBB-like gene cluster carrying the -158C→T mutation in Gp-promoter, which has been considered beneficial since the carriers of the haplotypes can have milder disease. [9, 37, 38]

In our study, it has been observed that hemoglobin levels of S, A2 and F in steady state SCA patients had no relationship with gender and these are in agreement with earlier studies. [30, 32] The study further revealed that there was no significant difference in HbA2 levels of HbAA, Hb SS and Hb SC and these are line with earlier findings [27, 30] but in contrary with the report of Majrashiet al. [33] however, the varying levels of HbA2 observed by the authors might be associated with different techniques employed and sample numbers.

The significantly lower HbF level in Hb AA subjects (control group) compared to Hb SS and Hb SC patients in this study has further confirmed the previous reports. [26, 27, 30, 33] Our study has further shown that there were no statistically significant differences in the HbA2 levels in male and female Hb SS patients compared to the controls (Hb AA subjects) and these are consistent with the earlier studies. [27, 30] However, HbF levels in male and female Hb SS patients in steady state compared to the controls (Hb AA subjects) showed statistically higher values and these are in support of previous findings. [33].

In conclusion, this study has determined the hemoglobin percentages of S, A2 and F in steady state, sickle cell disease patients using
HPLC. However, significantly higher level of HbF has been observed in steady state SCA patients irrespective of gender while HbA2 levels in Hb SS and Hb SC have shown no significant differences compared to Hb AA subjects. It is our believe that these findings will serve as guide to the physicians in the diagnosis and management of SCD patients in our environment.

References


