Association of HbA₂, HbF and HbS Values in Sickle Cell Disease Patients in Kano, Northwest Nigeria.

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Abstract

Background: Sickle cell disease accounts for over 60% of the world’s major haemoglobinopathies with an estimated 2-3% of Nigerians having HbSS. The aim of this study was to determine the association of hemoglobin levels of A₂, F and S in sickle cell disease patients. Methodology: A total of 140 sickle cell anaemia (SCA) patients, aged 1-33 years and 68, age-matched apparently healthy subjects 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 values of HbS, HbA₂ and HbF of 81.1±4.98%, 2.9±0.81% and 7.2±4.1% in steady state showed no statistically significant differences when compared to 81.88±4.0%, 2.47±0.9% and 7.25±3.66% in vaso-occlusive crisis, respectively (P>0.05). HbF level was significantly higher in HbSS patients (7.2±4.1%) than HbAS (1.9±1.84%) and HbAA (0.56±0.56%) subjects (P<0.05). HbS and HbF levels showed significant and strong negative relationship (r=-0.87, P=0.000) while HbS and HbA₂ levels showed significant and weak positive relationship (r=0.27, P=0.001) but HbA₂ and HbF revealed significant and weak negative relationship (r=-0.31, P=0.000). Conclusion: SCA patients in steady state and vaso-occlusive crisis showed no significant differences with respect to HbS, HbA₂ and HbF levels. HbF level is significantly higher in HbSS patients than HbAA and HbAS subjects. However, HbS and HbF levels showed significant and strong negative relationship in SCA. HbS and HbF levels are therefore recommended based on their strong relationship and their relevance in monitoring the progress of patients with sickle cell anemia.

Keywords: Association, Hemoglobin’s, Values Sickle Cell Disease.

Introduction

Sickle cell disease (SCD) is a chronic hemolytic disorder that is marked by the tendency of hemoglobin molecules within the red cell to polymerize and deform the red cell into a sickle (or crescent) shape resulting in characteristic vaso-occlusive events and accelerated haemolysis [1].

SCD is inherited in an autosomal recessive fashion either in the homozygous state or double heterozygous state. The homozygous state of inheritance is called sickle cell anemia (SCA) while other known SCD genotypes include HbSC disease, Sβ+ thalassaemia, HbSD Punjab disease, among others [1].

The hemoglobin disorders such as sickle cell anaemia and thalassaemias contribute significantly to the global toll of birth defects and these disorders occur most frequently in tropical countries. The disorders have been associated with mortality and disability in many countries because of population migration [2].

It has been estimated that 200,000 to 300,000 babies are born with sickle cell anaemia (SCA) in Africa each year and approximately 100,000 are born with this condition in the Middle East and India [3]. In Nigeria, carrier prevalence is about 20-30% [4, 5] while sickle cell disease affects about 2-3% of the Nigerian population of over 160 million [4].

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] while crisis refers to episodes of acute illness attributable to the sickling phenomenon in which there is a sudden deviation for the worse or a sudden exacerbation of symptoms and signs of patients with SCA who had been in stable condition [7].

In the diagnosis of sickle cell disease, quantification of HbA2, as well as a complete blood count (CBC), family history and clinical data help to establish the differential diagnosis between sickle cell anemia (HbSS) and Hbs/β+-thalassaemia [8,9].

Increased expression of HbA2 has been associated with compensation for the β-globin production inherent in β-haemoglobinopathies and thereby resulting in amelioration of the clinical severity of sickle cell disease [10]. However, both HbA2 and HbF have been proven to be effective in inhibiting intracellular deoxy-HbS polymerization [11].

High level of HbF has been associated with mild disease as it is uniformly dispersed within the red cell to retard sickling process [12] while high level of S of about 80-90% in the homozygous disease is associated with a worse disease [1]. However, a reciprocal relationship between HbA2 and HbF levels has been reported in previous studies [13, 14].

The importance of the levels of HbA2, HbF and Hbs cannot be overemphasized in the management of sickle cell disease patients. However, as a result of scanty information on these hemoglobin levels and their relationships, the study was designed to determine the levels of HbS, HbA2 and HbF in sickle cell anemia patients in steady state and vaso-occlusive crisis apart from correlating the values of HbS, HbA2 and HbF. It is our belief that the findings would assist the physicians in the management of sickle cell disease patients.

**Materials and Methods**

A total of one-hundred and forty sickle cell anaemia patients, aged 1-33 years, were recruited from Sickle Cell Clinic of Aminu Kano Teaching Hospital, Kano while sixty eight, age-matched, apparently healthy subjects of HbAA (control group) and HbAS were also studied between January, 2014 and February, 2016 after ethical approval from ethical committee of Aminu Kano Teaching Hospital and informed consent sought from the patients or guardians.

**Inclusion Criteria**

- Known patients with SCA as diagnosed by cellulose acetate electrophoresis at P<8.6 in the steady state, characterized by steady haematocrit and hemoglobin values over a given period of 2-3 clinic visits at 4-6 weeks intervals and a state of well-being without any symptoms or signs suggestive of crisis established by a careful history and complete physical examination [15].

- Known patients with SCA that were considered clinically to be in vaso-occlusive crisis based on the bone and joint pains or multiple sites of pain, requirement for analgesics and patients considering the episode as typical of crisis which necessitates hospital admission [16].

**Exclusion Criteria**

- Sickle cell disease patients who gave no consent
- Sickle cell anaemia patients with pregnancy

**Sample Collection**

Two milliliters (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. Haemoglobin levels of S, A2 and F were estimated from the blood samples using ion-exchange high performance liquid chromatography of Bio-Rad D-10 Dual Program according to manufacturer's instructions [17].

**Data Analysis**

Data were analyses using 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 one way ANOVA were used to assess the significant differences among two and three groups, respectively while correlation and regression analysis was employed to correlate the levels of hemoglobin types in SCA patients. The differences were considered to be statistically significant with P-values of less than 0.05.
Results
Hemoglobin levels of S, A₂ and F in sickle cell anaemia patients in steady state and vaso-occlusive crisis are represented in Table 1. The mean values of HbS, HbA₂ and HbF of 81.1 ± 4.98%, 2.9 ± 0.81% and 7.2 ± 4.1% in steady state showed no statistically significant differences compared to 81.78 ± 4.0%, 2.47 ± 0.9% and 7.25 ± 3.66% in vaso-occlusive crisis, respectively (P>0.05).

Table 2 shows the statistically significant differences of the mean values of HbA₂ of 2.96 ± 0.81%, 3.34 ± 0.69% and 2.9 ± 0.81%, and HbF of 0.56 ± 0.56%, 1.9 ± 1.84% and 7.2 ± 4.1% for HbAA, HbAS and HbSS subjects or patients, respectively (P<0.05).

Correlation between HbS and HbF levels in steady state SCA patients is shown in figure 1. HbS and HbF levels showed significant (P=0.000) and strong negative relationship (r= -0.87).

Figure 2 shows correlation between and HbA₂ levels in steady state SCA patients. HbS and HbA₂ levels showed significant (P=0.001) and weak positive association (r=0.27).

Correlation between HbA₂ and HbF levels in steady state SCA patients is revealed in figure 3. HbA₂ and HbF levels showed significant (P=0.000) and weak negative relationship (r=-0.31).

Table 1: Haemoglobin levels of S, A₂ and F in sickle cell anaemia patients in steady state and vaso-occlusive crisis

<table>
<thead>
<tr>
<th>Variable</th>
<th>HbSS (n=128)</th>
<th>Vaso-occlusive crisis</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbS (%)</td>
<td>81.1 ± 4.98</td>
<td>81.78 ± 4.0</td>
<td>0.6471</td>
</tr>
<tr>
<td>HbA₂ (%)</td>
<td>2.9 ± 0.81</td>
<td>2.47 ± 0.9</td>
<td>0.0837</td>
</tr>
<tr>
<td>HbF (%)</td>
<td>7.2 ± 4.1</td>
<td>7.25 ± 3.66</td>
<td>0.9676</td>
</tr>
</tbody>
</table>

Table 2: Comparison of HbA₂ and HbF levels in HbAA, HbAS and HbSS subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>HbAA (n=30) (Control group)</th>
<th>HbAS (n=38) (Steady state)</th>
<th>HbSS (n=128) (Steady state)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA₂ (%)</td>
<td>2.96 ± 0.81</td>
<td>3.34 ± 0.69*</td>
<td>2.9 ± 0.81</td>
<td>0.0112</td>
</tr>
<tr>
<td>HbF (%)</td>
<td>0.56 ± 0.56</td>
<td>1.9 ± 1.84**</td>
<td>7.2 ± 4.1**</td>
<td>0.0000</td>
</tr>
</tbody>
</table>

*HbA₂ level in HbAS compared to control (HbAA) (P=0.0406)
**HbF level in HbAS compared to control (HbAA) (P=0.0003)
**HbF level in HbSS compared to control (HbAA) (P=0.0001)
Discussion

Hemoglobin levels of S, A₂ and F in steady state sickle cell anemia in this study are in agreement with previous studies [18-20].

The study has further shown that there is no significant difference between the levels of HbF in steady state and vaso-occlusive crisis (VOC) and this is consistent with the earlier report [16]. However, Omoti and Omuemu [21] reported significantly higher HbA₂ level in sickle cell anemia in steady state compared to VOC and this is in contrary to our report which has shown no significant difference. The difference in HbA₂ levels may be associated with the methodology involved in the estimation of HbA₂ as Omoti and Omuemu [21] used a formula to calculate HbA₂ level while an accurate method, HPLC, was used in this study.

There is no earlier report to show the levels of HbS in SCA patients in steady state and VOC, however, this study has shown that there was no significant difference between HbS in steady state and VOC.

The study has further shown that there is no significant difference with respect to the levels of HbA₂ in HbAA (control group) and HbSS patients and this observation is in line with the previous report [22] but in contrary with the finding of Majrashi et al. [18]. However, the findings from different authors may be associated with the techniques employed and different sample numbers.

The study has also revealed that HbA₂ level is significantly higher in HbAS subjects than HbSS patients and HbAA subjects, and this has further confirmed the previous studies [20, 22]. However, low level of HbA₂ has been observed not to make meaningful clinical effect on HbS polymerization [23].

Our study has also shown that HbF level in HbSS patients is significantly higher than HbAA (control group) and HbAS subjects and these are in agreement with the previous authors [18, 20, 22]. However, high level of HbF has been associated with inhibition of deoxy-HbS polymerization and reduction of acute painful episodes, fewer leg ulcers, less osteonecrosis, less frequent acute chest syndromes, and reduced disease severity [23, 24].

The study has also displayed that HbS and HbF levels showed significant and strong negative relationship while HbS and HbA₂ levels showed significant and weak positive association. However, our observation on HbA₂ and HbF levels having significant and weak negative relationship is in agreement with the previous reports [13, 14] who observed reciprocal relationship between HbA₂ and HbF levels in acquired disorders when HbF levels are increased.

In conclusion, the study has shown that the development of SCA patients in steady state to vaso-occlusive crisis is not associated with the levels of HbS, HbA₂ and HbF. However, HbF level is significantly elevated in HbSS patients than HbAA and HbAS subjects.

HbS and HbF levels, and HbA₂ and HbF levels have shown negative associations while HbS and HbA₂ levels have displayed positive relationship.

However, it is recommended that HbF and HbS levels be determined in patients with SCA since they are strongly negatively correlated in this study apart from their relevance in the management of patients with sickle cell disease.
References


