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Chromatographic analysis of Hb S for the diagnosis of various sickle cell disorders in Pakistan

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Abstract Sickle cell disease remains a relatively obscure theme in research on haemoglobinopathies in Pakistan. Limited data is available regarding its prevalence in the country. The objective of our study was not only to estimate the frequency of different sickle cell diseases but also to provide quantitative estimation of haemoglobin S and other haemoglobin variants using an automated high-performance liquid chromatography (HPLC) system. For this purpose, we retrospectively evaluated the results of HPLC performed on all patients with suspected haemoglobinopathies during the years 2005 and 2006. Information derived from various sources was used to identify a particular genotype by analysing each sample containing Hb S with respect to haemoglobin, red cell indices and levels of various associated haemoglobin variants. Analysis of 15,699 samples identified 302 patients with Hb S (1.92%). The genotypes identified included Sβ0 (46.7%), SS (19.2%), SA (11.6%), Sβ+ (8.6%) and SD (2.3%). Thirty-five cases could not be categorised and were labelled 'unclassified'. Majority of the patients (62.3%) were below the age of 18 years. Balochistan, which is the largest province based on the area, yielded the highest number of patients (n=140). In the Sβ0 group, the mean haemoglobin and Hb S were lower in children compared to adults (p value of 0.001 and 0.016, respectively). We conclude that sickle cell disorders are prevalent in Pakistan to a significant extent, being concentrated in certain areas of the country. We present the first report of various haemoglobin S genotypes from our population. It is hoped that it will act as a database to characterise the same for our population.

Keywords High-performance liquid chromatography · Sickle cell disorders · Hb S · Haemoglobinopathy

Introduction

In 1948, Linus Pauling and Harvey Itano employed the then new technique of haemoglobin electrophoresis to prove that haemoglobin molecules in patients suffering from sickle cell anaemia have a different electrical charge compared to that of normal individuals [1]. Eight years later, Vernon Ingram and J.A. Hunt first sequenced sickle haemoglobin (βs) and discovered the single base substitution of glutamic acid by valine in the β globin gene [2]. These were the first instances of the biochemical diagnosis of sickle cell disease. Almost six decades after Pauling's initial discovery, sickle cell anaemia still remains a widely researched theme with upcoming novel approaches to diagnosis and management.

The diagnosis of sickle cell anaemia is gaining importance, as developing nations like Pakistan undergo a transition from an era of communicable to non-communicable disease [3]. Haemoglobinopathies are relatively common in Asian and African countries, probably due to their social customs and consanguineous marriages [4]. Indeed, the Hb S allele is highly prevalent in the Middle East, Africa and the Indian subcontinent [5]. Miller et al. conducted a survey of
pre-school children in the United Arab Emirates (UAE) and discovered the frequency of sickle cell trait to be 4.6% [6]. Baysal [7] examined the beta globin genes of 313 patients with beta thalassemia and found that 21.9% of them were also carriers for the $\beta^+$ chromosome. The national register of haemoglobinopathies of Oman reports a carrier rate of 10% for sickle cell anaemia, which is very high compared to the neighbouring UAE [8].

Limited data is available regarding the prevalence of sickle cell disease in Pakistan. Some early research focusing on the incidence and clinical manifestation exists. In 1990, Kazmi and Rab reported eight cases of sickle cell disease, which was presented to the medical ward. They claimed that the trait, though common in our population, frequently escapes detection [9]. Moreover, they evaluated their patients through haemoglobin electrophoresis, which is unable to differentiate haemoglobin S from D at alkaline pH [10–12]. Recently, a population-based survey at Karachi University estimated the frequency of sickle cell disease to be 5.1% among the different ethnic groups in the city [13]. However, this data is limited to one major city, and no country-wide initiative has been launched to quantify the prevalence of the sickle cell trait and to identify the homozygotes for this mutation. These authors used molecular studies to quantify Hb S in their patients. Although very accurate, this technique is not the best initial diagnostic test due to the availability of this technique in very few centres across the country.

We have used high-performance liquid chromatography (HPLC) to diagnose and quantify the frequency of various sickle cell disorders in our sample. To the best of our knowledge, there are no other publications pertaining to the diagnosis of Hb S from Pakistan using HPLC.

Materials and methods

From January 2005 to December 2006, 15,699 samples from patients with suspected haemoglobinopathies were analysed in the clinical laboratory of The Aga Khan University for determination of haemoglobin variants. The patients were referred by various physicians from within and outside the hospital. Since the laboratory acts as a referral laboratory for the country, we were able to collect samples from 54 blood collection centres within the city and 82 similar centres from the entire country.

Samples were collected after institutional Ethical Review Committee approval and informed consent from the patients. No information about the patient's identity was included in the data entry, and individual records were accessed using patient record numbers. Access to the data was restricted only to the authors, and no reference was made to the patient's identity during data analysis or manuscript formulation.

Five millilitres of blood was collected from each patient in ethylene diaminetetraacetate microvets (BD, Becton Dickinson and Company, New Jersey, USA) after informed consent. The samples were received and cooled at 4°C by fast transportation in our lab and were analysed within 2 days of arrival.

Cation-exchange HPLC is favoured over haemoglobin electrophoresis as a reliable method for the initial screening of haemoglobin variants [12]. It is also useful for the screening of various haemoglobinopathies including thalassemia [14] and neonatal diagnosis of sickle cell disease [15]. We used the Bio-Rad Variant $\beta$ Thalassemia Short program (Bio-Rad Laboratories Inc., Hercules, CA, USA) for haemoglobin quantification. This automated system utilises the principle of cation exchange HPLC with detection at double wavelength (415 and 690 nm) [16]. The instrument is user friendly and analysed each sample for a period of 5–6 min, with various positively charged haemoglobin molecules eluting at different times depending on their affinity for anion-coated resin columns. We used a retention time of 4.5 min to identify Hb S [17, 18].

In addition, haemogram was performed on each sample by Coulter Gen-S (Coulter Electronics, Fullerton, CA, USA), and peripheral film stained with Leishman was examined by experienced technologists and haematologists.

We used information derived from various sources [19, 20] to identify a particular genotype by analysing each sample containing Hb S with respect to haemoglobin, red cell indices and levels of various associated haemoglobin variants (Hb F, Hb A, Hb A$_2$ and any other; Table 1).

Statistical analysis

All statistical analyses were performed using SPSS Version 14.0 (SPSS Inc., Chicago, IL, USA). The laboratory data of 2005 and 2006 was obtained from the Information Technology Department of the hospital. All samples with Hb S were identified, and the relevant complete blood count findings were added for each patient, if available. This was verified by two investigators to avoid any errors. The patients were categorised in different groups based on the HPLC values of haemoglobin variants shown in Table 1, and descriptive statistics were calculated for each of the groups. These included the mean±one standard deviation (±1 SD) for all continuous data and the maximum and minimum values for each variable. The median age was also computed for all groups as shown in Table 2. Univariate analysis was performed using the independent samples $t$ test, where means were compared between children and adults and between adult males and females in each category. A $p$ value of less than 0.05 was considered to be statistically significant for all analyses.
Results

In all our evaluated specimens, chromatography showed that Hb S was identified in 302 patients (1.92%). Overall, there were 193 males and 109 females with ages ranging from 1 to 56 years (median age=13 years). The different genotypes detected among our population along with demographics are given in Table 2. The most frequent genotype was S/β^0, being identified in 141 (46.7%) patients, while SS was the second most common as it was seen in 58 (19.2%) patients. Majority of the patients (61.4%) were diagnosed below the age of 18 years.

Balochistan, one of the largest provinces of our country, yielded the highest number of patients with sickle cell disease contributing 140 (46.4%) cases with Hb S to our study. Similarly, 100 (33.1%) cases were diagnosed from Sind, yet another large province inhabited by similar ethnic groups as Balochistan.

Haematological parameters and various genotypes identified in our patients are summarised in Table 3, while red cell indices are given separately in Table 4.

Sickle cell disease

Of the 58 patients categorised as having sickle cell disease (SS), more children than adults were diagnosed, with a ratio of 2.2:1. HPLC showed the presence of haemoglobins S, F and A2, with A being entirely absent as shown in Table 3. No significant difference was noted in the means of the haemoglobin variant between adult males and females or between children and adults. Hb A2 was below 4.00 in all groups. The haemoglobin concentration reported in adults ranged from 6.30 to 12.10 g/dl, while the same in children was from 5.10 to 11.40 g/dl (reference range for men: 13.7–16.3 g/dl and women: 11.1–14.5 g/dl). Red cell indices were normal in all the patients as shown in Table 4. RDW is generally increased, corresponding to the degree of anaemia. Total leukocyte count and platelet count were in the range of 6.00 to 16.40×10^9/l and 211 to 556×10^9/l, respectively (data not shown) in adults, while the same for children were lower and ranged from 7.40–38.70×10^9/l and 176.0–800×10^9/l, respectively. Sickle cells were identified on peripheral film along with anisocytosis and poikilocytosis, polychromasia, fragmented and nucleated RBCs.

Sickle cell trait

Similar to homozygous SS, it was observed that majority of the patients diagnosed as sickle cell trait (SA; n=35) were below 18 years of age (60%). The haemoglobin concentration was subnormal in all the groups, while mean cell volume (MCV) and mean cell haemoglobin (MCH) were reduced in children. Blood film showed hypochromic and microcytic red cells in eight patients. The proportion of haemoglobin S was quite variable, ranging from 29.6% to 43.3% among all groups. Hb A2 was up to 4.0 in SA.

Table 1 Usual haematological characteristics in adults with various sickle cell disorders [19, 20]

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Hb (g/dl)</th>
<th>MCV (fl)</th>
<th>Hb S (%)</th>
<th>Hb A (%)</th>
<th>Hb A2 (%)</th>
<th>Hb F (%)</th>
<th>Any other</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS</td>
<td>6–10</td>
<td>70–100</td>
<td>90–95</td>
<td>0</td>
<td>2–4</td>
<td>5–10</td>
<td>10–20</td>
</tr>
<tr>
<td>SA</td>
<td>Normal</td>
<td>Normal</td>
<td>30–45</td>
<td>50–65</td>
<td>Normal(&lt;3.5)</td>
<td>2–5</td>
<td></td>
</tr>
<tr>
<td>Sβ^0</td>
<td>7–11</td>
<td>60–80</td>
<td>&gt;50</td>
<td>0</td>
<td>4–5.6</td>
<td>5–15</td>
<td></td>
</tr>
<tr>
<td>Sβ^-</td>
<td>9–12</td>
<td>60–80</td>
<td>&lt;50</td>
<td>5–30</td>
<td>&gt;3.5</td>
<td>&lt;20</td>
<td></td>
</tr>
<tr>
<td>S/β^0</td>
<td>10–12</td>
<td>76–83</td>
<td>Not available</td>
<td>Not available</td>
<td>1.9–2.3</td>
<td>15–25</td>
<td></td>
</tr>
<tr>
<td>S/HPFH</td>
<td>&gt;12</td>
<td>68–88</td>
<td>60</td>
<td>0</td>
<td>1.1–2.2</td>
<td>20–30</td>
<td></td>
</tr>
<tr>
<td>S/D</td>
<td>5–10</td>
<td>Normal or ↑</td>
<td>40</td>
<td>0</td>
<td>2–3</td>
<td>2.5–5</td>
<td>D Punjab: 50</td>
</tr>
<tr>
<td>S/Hb Lepore</td>
<td>11</td>
<td>↓</td>
<td>75</td>
<td>0</td>
<td>2</td>
<td>3.5–40</td>
<td>Lepore: 10</td>
</tr>
<tr>
<td>SS −α/α,</td>
<td>8.6</td>
<td>↓</td>
<td>80–90</td>
<td>0</td>
<td>3.3–3.8</td>
<td>2–20</td>
<td></td>
</tr>
<tr>
<td>SS −α−α,</td>
<td>9.2</td>
<td>↓</td>
<td>80–90</td>
<td>0</td>
<td>3.3–3.8</td>
<td>2–20</td>
<td></td>
</tr>
<tr>
<td>S/C</td>
<td>10–14</td>
<td>Normal or ↑</td>
<td>40–50</td>
<td>0</td>
<td>Not available</td>
<td>1.1–3.3</td>
<td>Hb C: 40–50</td>
</tr>
</tbody>
</table>

Table 2 Frequencies and demographics of various sickle cell disorders (n=302)

<table>
<thead>
<tr>
<th></th>
<th>SS (%)</th>
<th>SA (%)</th>
<th>Sβ^0 (%)</th>
<th>Sβ^- (%)</th>
<th>SD (%)</th>
<th>Unclassified (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients, n (%)</td>
<td>58 (19.2)</td>
<td>35 (11.6)</td>
<td>141 (46.7)</td>
<td>26 (8.6)</td>
<td>7 (2.3)</td>
<td>35 (11.6)</td>
</tr>
<tr>
<td>Males, n (%)</td>
<td>34 (58.6)</td>
<td>16 (45.7)</td>
<td>103 (27.0)</td>
<td>16 (61.5)</td>
<td>4 (57.1)</td>
<td>14 (40.0)</td>
</tr>
<tr>
<td>Females, n (%)</td>
<td>24 (41.4)</td>
<td>19 (54.3)</td>
<td>38 (73.0)</td>
<td>10 (38.5)</td>
<td>3 (42.9)</td>
<td>21 (60.0)</td>
</tr>
<tr>
<td>Age in years (median)</td>
<td>11</td>
<td>13</td>
<td>14</td>
<td>19</td>
<td>16</td>
<td>14</td>
</tr>
</tbody>
</table>
Sickle cell/β thalassemia syndromes

Sickle cell/β thalassemia (including Sβ0 and Sβ+) encompasses the largest group in our study, comprising of 167 patients of whom 103 were children. No Hb A was detected in Sβ0, while it ranged from 9.10% to 58.4% in children and 11.40% to 53.50% in adults with Sβ+. Hb S and Hb F were elevated in all cases, as indicated in Table 3. The mean haemoglobin and Hb S values were lower in children compared to adults in the Sβ0 group (p=0.016 and p=0.001, respectively). Surprisingly, the haemoglobin concentration did not show much difference between Sβ0 and Sβ+. Red cell indices were reduced in all cases. Peripheral film findings were more severe in Sβ0 than in Sβ+, with

Table 4 Red cell indices in various haemoglobin S genotypes (n=302)

<table>
<thead>
<tr>
<th>Hb S genotype</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Hb (g/dl)</th>
<th>MCV (fl)</th>
<th>MCH (pg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS</td>
<td>&lt;18 Both</td>
<td>40</td>
<td>73.55±8.83</td>
<td></td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>&gt;18 M</td>
<td>8</td>
<td>74.43±5.25</td>
<td>2.86±0.42</td>
<td>19.78±5.55</td>
</tr>
<tr>
<td></td>
<td>&gt;18 F</td>
<td>10</td>
<td>78.24±7.53</td>
<td>2.73±0.52</td>
<td>19.03±7.58</td>
</tr>
<tr>
<td>SA</td>
<td>&lt;18 Both</td>
<td>21</td>
<td>35.47±4.39</td>
<td>30.00±0.66</td>
<td>0.65±1.22</td>
</tr>
<tr>
<td></td>
<td>&gt;18 M</td>
<td>2</td>
<td>39.95±4.74</td>
<td>3.55±0.21</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>&gt;18 F</td>
<td>12</td>
<td>36.20±3.41</td>
<td>3.27±0.80</td>
<td>0.375±0.88</td>
</tr>
<tr>
<td>Sβ0</td>
<td>&lt;18 Both</td>
<td>90</td>
<td>70.91±8.79</td>
<td>5.44±0.84</td>
<td>23.58±9.21</td>
</tr>
<tr>
<td></td>
<td>&gt;18 M</td>
<td>41</td>
<td>74.43±8.28</td>
<td>5.50±0.88</td>
<td>21.97±13.46</td>
</tr>
<tr>
<td></td>
<td>&gt;18 F</td>
<td>10</td>
<td>75.10±7.31</td>
<td>5.23±0.65</td>
<td>19.67±7.63</td>
</tr>
<tr>
<td>Sβ+</td>
<td>&lt;18 Both</td>
<td>13</td>
<td>47.95±15.14</td>
<td>5.58±1.91</td>
<td>10.96±6.89</td>
</tr>
<tr>
<td></td>
<td>&gt;18 M</td>
<td>7</td>
<td>50.07±12.06</td>
<td>4.99±0.43</td>
<td>13.07±7.57</td>
</tr>
<tr>
<td></td>
<td>&gt;18 F</td>
<td>6</td>
<td>51.43±19.80</td>
<td>5.08±0.70</td>
<td>12.65±11.00</td>
</tr>
<tr>
<td>SD</td>
<td>&lt;18 Both</td>
<td>4</td>
<td>29.50±12.13</td>
<td>1.53±1.10</td>
<td>25.03±17.83</td>
</tr>
<tr>
<td></td>
<td>&gt;18 M</td>
<td>1</td>
<td>46.40</td>
<td>2.80</td>
<td>4.70</td>
</tr>
<tr>
<td></td>
<td>&gt;18 F</td>
<td>2</td>
<td>45.95±14.21</td>
<td>2.30±0.28</td>
<td>8.35±11.81</td>
</tr>
<tr>
<td>Unclassified</td>
<td>&lt;18 Both</td>
<td>21</td>
<td>38.06±17.94</td>
<td>3.14±0.60</td>
<td>12.70±10.29</td>
</tr>
<tr>
<td></td>
<td>&gt;18 M</td>
<td>6</td>
<td>29.58±19.75</td>
<td>2.47±0.46</td>
<td>9.13±9.15</td>
</tr>
<tr>
<td></td>
<td>&gt;18 F</td>
<td>8</td>
<td>29.42±10.71</td>
<td>3.04±0.98</td>
<td>3.65±6.32</td>
</tr>
</tbody>
</table>

Unclassified The samples that cannot be adequately categorised in any other group, M males, F females, Both males and females

Hb reference range for men: 13.7–16.3 g/dl and women: 11.1–14.5 g/dl. Reference range for MCV: 76–96 fl, MCH: 26–32 pg

Unclassified The samples that cannot be adequately categorised in any other group, M males, F females, Both males and females, NA not available

*a Only one sample of complete blood count was available in this group.
classical sickle cells seen in Sβ⁺ along with hypochromic, microcytic cells and poikilocytes.

Sickle cell/Hb S D

Compound heterozygosity for Hb S and Hb D was detected in seven patients, including four children and three adults. Hb D formed a higher proportion (43.95%) of total haemoglobin than Hb S (29.5%) in children, while the two were nearly similar in concentration in adults. The haemoglobin concentration and red cell indices could not be retrieved from our computerised database.

Unclassified

There were 35 patients who could not be characterised correctly into any of the categories. These patients had varying proportions of haemoglobin variants on electrophoresis including Hb S, Hb A, Hb A₂, Hb F with Hb D observed in five patients (Table 3). Haemoglobin was subnormal in both children and adults (Table 4). The authors believe that these patients represent post transfusion SS or Sβ⁰.

Discussion

The frequency of sickle cell disorders in our study, as identified by HPLC, was 1.92%. HPLC has been found to be a reliable, reproducible and in most cases, a superior method to conventional electrophoresis in identifying haemoglobin variants [21]. This advantageously rapid detection was used by Frietsch et al. [22] in the monitoring of changes in the proportion of Hb S variants during surgery of sicklers. Although the initial cost of the equipment is unaffordable by most laboratories in Pakistan, this is largely offset by increased use and fast throughput of instrument [11, 23]. The cost per test was $5.0, as calculated by estimating consumables and labour charges. An added advantage of HPLC is the detection of co-existing haemoglobinopathies for which multiple gel electrophoresis or PCR tests would be required. It was for these reasons that the clinical laboratories of Aga Khan University Hospital switched from gel electrophoresis to HPLC in 2001.

The prevalence of sickle cell disorders in Pakistan has been estimated as being less than 1% [20], with the carrier rate of 0.5% to 1% [24]. However, isolated reports have shown the carriage to be as high as 10% in Balochistan [4]. There is a dearth of literature on any country-wide initiative to confirm or challenge this statistic. In sharp contrast to this, sickle cell prevalence of up to 35% has been reported in certain areas of neighbouring India [20, 25]. Our study demonstrated a frequency of 1.92%, which is similar to what has been reported earlier from this region. Ghani et al. reported Hb S in 5.1% of 200,000 anaemic patients evaluated by molecular techniques in multi-ethnic population of Karachi in 2002 [13]. The higher frequency reported by him contrasts with our results. This may be attributed to the difference in patient distribution, as we analysed samples from all over country.

A majority of our study subjects were children (61.4% vs. 38.6% adults). This pattern was uniformly observed in all genotypes. This was expected among homozygotes and severely affected compound heterozygotes (S/β) as they usually manifest themselves at a younger age [20]. It is interesting that boys were diagnosed at the age of 2 years or less, while girls were 9 years or older. This may represent our social and cultural norms where boys are taken better care of and usually receive medical attention earlier than girls. Again, it is fascinating to note that twice as many boys were diagnosed with the disorder than girls. This gender-biased data could be due to the reason mentioned previously or attributed to milder manifestations of the Arab–Indian haplotypes among females as reasoned by Steinberg [26].

The clustering of sickle cell disease in Balochistan and Sind is no surprise. It is known that spontaneous mutation in the β globin gene has occurred at least four times in history leading to five major β⁰ globin gene haplotypes [20]. These haplotypes are identified by their associations with DNA polymorphisms. There are four major African haplotypes, namely the Senegal, Benin, Bantu and Cameroon haplotypes. The fifth one is the Arab–Indian haplotype and it is said to have originated in the Indus Valley civilization, the ruins of which are found in present day Harappa and Taxila in our country [23]. The Arab–Indian haplotype is found among tribes living in the eastern oasis of Saudi Arabia and many tribes in India and Oman. The ancient trade routes between India and Europe via the Middle East, especially Oman, Iraq and Yemen, explains this gene migration from its site of origin. The influx of Balochis in Oman occurred between the eighteenth and twentieth centuries, and the Arab–Indian haplotype is prevalent in the descendants of these immigrants [5]. We, therefore, conclude that β⁰ globin gene exists in Balochi tribes [20, 26], and theoretically, they should have the Arab–Indian haplotype.

The present study generates some data as regards various Hb S genotypes. We found compound heterozygotes with sickle/β thalassemia as the major group among various sickle cell disorders, although an incidence of less than 10% have been reported by some [19], with a preponderance of β⁺ phenotype. Our conflicting results may represent the simultaneous high prevalence of β thalassemia in our population [27]. Sickle cell anaemia and trait were diagnosed in patients presenting with low haemoglobin. It
is quite possible that patients with SA genotype represent the tip of iceberg, as many individuals with normal haemoglobin will never undergo screening tests. Few patients with sickle-Hb D were also diagnosed, which was quite predictable as Hb D is fairly common in Pakistanis [20].

All patients with SS had normochromic normocytic anaemia with circulating sickle cells and nucleated red cells, their number corresponding to the severity of anaemia. Adults in this category also had elevated white cell and platelet counts, a finding that may be attributed to hyposplenism.

It is surprising that patients with SA uniformly showed low haemoglobin with low MCV and MCH with the degree of anaemia comparable to SS, although such patient are usually reported to be asymptomatic with normal haemoglobin and red cell indices [19, 20]. The reason for this discrepancy may be the presence of co-existing α-thalassemia [20] and/or iron deficiency [28]. As we did not incorporate DNA and iron studies in our study design, it is very difficult to conclude on this aspect with any level of confidence. However, several studies have demonstrated the prevalence of α-thalassemia [29] and significant iron deficiency anaemia among Pakistanis [13, 30]. Patient with sickle/beta thalassemia also had anaemia with hypochromic microcytic indices and haemoglobin concentration less than that seen in SS and SA. Similar findings have been observed by others as well [20].

Hb F was significantly higher in SS category as compared to previous information (refer to Table 1 and Table 2). The reasons may be the existence of Arab–Indian haplotypes in our population as values in the range of 10–25% has been reported in these [31] or associated polymorphisms with hereditary persistence of foetal haemoglobin (HPFH). Since Hb F is a potent inhibitor of polymerization of deoxyhaemoglobin S, its high percentage would lead to fewer clinical manifestations [32]. This might be the reason that SS was identified less frequently in our population since Hb F as high as 29% was observed in our study.

Elevated Hb A2 was observed in our patients with sickle cell trait. This is in agreement with previously published reports. The reasons for this may be multifactorial such as interaction of excessive α chains with δ to synthesise more Hb A2 [16], co-elution of post-translationally modified carbamylated α-globin with Hb A2 [33], sample aging [34] or the presence of glycosylated haemoglobin [35]. We did not encounter any case of Hb S with Hb Lepore, HPFH or Hb C in our study. This may be due to the absence of anaemia in the first two conditions [20], which escaped detection in our evaluation as all patients in our study were anaemic. Compound heterozygotes with Hb C were not observed since this variant is infrequent in Pakistan.

Limitations

Our study design was limited by several factors. Being a retrospective analysis of HPLC finding for the years 2005 and 2006, clinical details, particularly ethnicity, were not known. Also, complete blood counts could not be retrieved for many patients. Iron studies and other confirmatory tests for the diagnosis of Hb S variants were not performed. However, several studies that were performed to validate the automated HPLC method for quantification of Hb S have found an excellent correlation with other techniques [36, 37].

The clinical laboratories of the Aga Khan University serve as a referral centre for the diagnosis of various haemoglobinopathies. As we received blood samples from the entire country, the authors believe that this study accurately portrays the distribution of the various Hb S genotypes in our population.

Conclusion

Sickle cell disease is prevalent in Pakistan, being significantly concentrated in certain areas of the country. We present the first report of various sickle cell disorders from Pakistan. We hope that it will act as a database to characterise the Hb S genotypes in our population. However, there is a need to carry out prospective studies to elucidate the true dimensions of the problem.

References