Thalassaemia and glucose-6-phosphate dehydrogenase deficiency in sickle-cell disorder patients in Taiz, Yemen

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ABSTRACT A pilot study was conducted to determine the prevalence and haematological characteristics of the interaction between thalassaemia or/and glucose-6-phosphate dehydrogenase (G6PD) deficiency in patients with sickle-cell disorder (SCD) in Taiz city, Yemen, where the prevalence of sickle-cell trait (HbAS) is 8.2%. Blood samples were collected from 31 SCD patients. Complete blood count and haemoglobin electrophoresis, G6PD activity and serum ferritin were determined. Thalassaemia was found in 6 patients (19.4%) and G6PD deficiency (6 mild and 1 severe) was detected in 7 patients (22.6%). The frequency of thalassaemia and/or G6PD deficiency with SCD was high and this may have an effect on the severity of the clinical course of SCD in Taiz. The study should be repeated with DNA analysis to define the nature of the globin gene defect and to clarify its role in the severity of SCD.
Introduction

Sickle-cell disorder (SCD), thalassaemia, and glucose-6-phosphate dehydrogenase (G6PD) deficiency are inherited disorders causing haemolytic anaemia in humans. SCD and thalassaemia are the most common genetic disorders worldwide [1].

The clinical manifestations of SCD are due to the tendency for the haemoglobin (Hb) S variant to polymerize and deform red blood cells into the sickle shape under deoxygenated conditions. The homozygous state of HbS is the most common form of SCD [1], but its interaction with thalassaemia and certain variant haemoglobins also leads to sickling and may affect its clinical picture. Interaction of SCD with thalassaemia and G6PD deficiency has been reported in many studies [2,3], since all of these disorders share the characteristic that their carriers have partial protection against malaria infection. The high prevalence of these genetic defects in certain human populations may be due to the selective pressure of malaria worldwide [1].

Yemen is located on the south-west of the Arabian peninsula and has a population of 21 million [5]. It is estimated that the annual number of cases of malaria infection in Yemen are around 3 million [6]. The overall prevalence of sickle-cell trait (HbAS) in Yemen is 2.2%, with a higher frequency in the mid-western region where Taiz city is located than in the central and eastern areas [7]. The prevalence of thalassaemia trait in Yemen is 13.3% (β-thalassaemia trait 4.4%, α-thalassaemia trait 8.9%) [8]. There are no previous reports on the occurrence of G6PD deficiency or thalassaemia in Yemeni SCD patients, except one showing that the coexistence of α-thalassaemia in 26 Yemeni children with SCD living in Riyadh was 57.7% [9].

The present pilot study was conducted to determine the prevalence and haematological characteristics of the interaction between thalassaemia or/and G6PD deficiency in patients with SCD in Taiz, where the prevalence of HbAS is 8.2% [7]. Such studies are essential to evaluate how these conditions impact on the severity of the clinical features of SCD and to clarify their role in childhood mortality and morbidity.

Methods

Between November and December 2007, blood samples were collected from 31 patients with SCD attending the Yemen Society for Thalassaemia and Genetic Blood Disorders in Taiz city (18 males, 13 females, aged between 6 months to 18 years) who came from different territories of Taiz governorate (Table 1).

Two venous blood samples (1 collected into EDTA, 1 clotted) were collected from each patient. The first one was used for determination of complete blood count (CBC), using the Sysmex KX-21N 2 haematology analyser (Sysmex Corporation); separation of haemoglobin variants, using high-performance liquid chromatography (HPLC) (Bio-Rad); G6PD activity, using a quantitative assay kit (Randox Laboratories); and sickling test, using the sodium metabisulfate method. The second sample was used for estimation of the serum ferritin level, using the AxSym fluorescence microparticle enzyme immunoassay method (Abbott). Blood films were stained with Leishman stain.

Presence of SCD was defined by a positive sickling test and HbS concentration > 50% of the total Hb by high-pressure liquid chromatography. Thalassaemia was indicated by microcytic hypochromic red cells: mean corpuscular volume (MCV) < 80 fL and/or mean corpuscular haemoglobin (MCH) < 27 pg, after adjustment of the MCV/MCH for each age group with normal or high serum ferritin. Normal range for serum ferritin was 20–260 ng/mL; high serum ferritin was > 260 µg/L; low serum ferritin was < 20 µg/L.

Patients were defined as:

- SCD + thalassaemia: coexistence of SCD with microcytic hypochromic red cells with normal or high serum ferritin.
- SCD + G6PD deficiency: coexistence of SCD with G6PD deficiency.
- SCD + thalassaemia + G6PD deficiency: coexistence of SCD with microcytic hypochromic red cells, normal serum ferritin and G6PD deficiency.

High level of fetal Hb (HbF) was > 20%. A high nucleated red blood cell (RBC) count was ≥ 1 and high white blood cell (WBC) count was ≥ 10 000/L.

Results

All the samples showed a positive sickling test. No HbA was detected in any of them. The distribution of the cases by sex, rate of SCD, rate of SCD + thalassaemia and rate of SCD + G6PD deficiency by territory of Taiz city is presented in Table 1. A total of 19 cases (61.3%) showed SCD without thalassaemia or G6PD deficiency, 6 (19.4%) were considered as SCD + thalassaemia and 7 (22.6%) were SCD + G6PD deficiency (6 cases of mild > 20–< 65% and 1 case of severe G6PD deficiency < 20%). Only 1 SCD case (3.2%) was considered as SCD + thalassaemia + G6PD deficiency.

There were 30 SCD samples (96.8%) that showed high serum ferritin: 24 had levels between 260 and 1000 µg/L and 6 had levels > 1000 µg/L. Only 1 sample showed serum ferritin (53 ng/mL) within the normal range. No SCD samples with low serum ferritin were reported in this study.
Of the SCD samples, 30 showed nucleated RBCs and 12 (38.7%) showed a high nucleated RBC count in the blood film (between 10%–58%). Of these, 6 cases had nucleated RBCs > 20% and 2 cases > 50%.

The mean values of the haematological parameters in cases of SCD and SCD + thalassaemia or SCD + G6PD deficiency are shown in Table 2. The cases of SCD + G6PD deficiency had slightly higher total Hb than the cases of SCD or SCD + thalassaemia. The SCD cases had the lowest total Hb, the highest WBC count and the highest percentage of nucleated RBCs. However, the WBC count was high in SCD, SCD + thalassaemia and SCD + G6PD deficiency.

The 4 SCD cases with Hb F > 20% (2 with SCD, 1 with SCD + thalassaemia, 1 with SCD + G6PD deficiency) showed higher total Hb and lower WBC counts than the 27 SCD cases with Hb F< 20% (Table 3).

Discussion
This study showed that in Taiz region the prevalence of thalassaemia among SCD patients was 19.4%, whereas the prevalence of mainly mild G6PD deficiency with SCD was 22.6%. Only 1 of the 31 samples showed an association of SCD with both thalassaemia and G6PD deficiency. Absence of HbA in all the samples of this study indicated that these cases could be homozygous sickle, sickle cell/β-thalassaemia, homozygous sickle cell/β-thalassaemia or homozygous sickle cell/α-thalassaemia.

The diagnosis of thalassaemia was based on low MCV < 80 fl and/or MCH < 27 pg, after adjustment of the MCV/MCH for each age group with normal or high serum ferritin. Using the HbA2 level for distinguishing between α- and β-thalassaemia traits in this study was not appropriate because its level may overlap, since HbA2 may be raised in the presence of HbS, SCD/α-thalassaemia and the heterozygous compound of S/β-thalassaemia [10,11].

Of the SCD samples, 12 showed a high percentage of nucleated RBCs: 6 had > 20% nucleated RBCs and 2 had > 50%. This high number of cases with nucleated RBCs reflects the level of hyper-erythropoiesis, suggesting a high presence of young RBCs which may mask the diagnosis of G6PD deficiency type A(–), since these immature RBCs are rich with G6PD [12]. Therefore, the prevalence of G6PD deficiency interaction with SCD may have been underestimated in this study or the detection of severe G6PD deficiency may have been masked in patients with SCD.

Of all the samples 30 showed high serum ferritin > 260 ng/mL, 6 of which had serum ferritin > 1000 ng/mL, suggesting that these patients were either not using the chelating agent...
(deferoxamine mesylate) or used it improperly. Chronic infection may also play a role in this as the mean WBC count was high for these SCD patients, although an increased WBC count in SCD may be partially due to a shift of granulocytes from the marginated pool of cells to the circulating pool. In this study normal and high serum ferritin levels were considered as indicators for the absence of iron deficiency.

The slightly higher RBC counts in cases of SCD + thalassaemia than cases of SCD + SCD + G6PD deficiency is in accordance with the increased RBC counts in thalassaemia trait which may support the diagnosis of thalassaemia [13]. The slightly higher WBC counts and lower level of total Hb in SCD + thalassaemia cases suggests that coexistence of thalassaemia + SCD worsens the severity of SCD.

This study showed a correlation between a high level of HbF (> 20%) and higher total Hb and lower WBC counts than those with low HbF (< 20%), which may indicate that a high level of HbF has a beneficial effect on the clinical picture of SCD, as previously reported in other studies [14].

The slightly higher total Hb in cases of SCD + G6PD deficiency than those with SCD and SCD + thalassaemia may support the theory of simultaneous inheritance of G6PD deficiency and sickle-cell anaemia. This is because in G6PD deficiency, SCD increases the number of reticulocytes that are rich in the G6PD enzyme, and in SCD, G6PD deficiency increases the numbers of young RBCs and decreases the old RBCs which decrease the irreversibly sickled erythrocytes [12].

This study represents the first attempt to determine the prevalence of interaction of thalassaemia and/or G6PD deficiency in patients with SCD in Taiz. The prevalence of thalassaemia in SCD was 19.4%, less than that found previously in Yemeni children living in Riyadh by El-Hazmi et al. [9]. Further investigation is warranted using DNA techniques in order to accurately diagnose those who are found to have microcytic red cells and to study the nature of the mutations involved for predicting the likely severity of the clinical course of SCD in association with thalassaemia and/or G6PD deficiency. A larger study is required to identify the effect of the α- or β-thalassaemia, G6PD deficiency and the other genetic Hb defect disorders on the severity of the clinical picture of SCD in Yemen.

<table>
<thead>
<tr>
<th>Haematological parameters</th>
<th>SCD (n = 19) (age 1.5–18 yrs)</th>
<th>Mean (SD)</th>
<th>SCD + thalassaemia (n = 6) (age 0.5–9 yrs)</th>
<th>Mean (SD)</th>
<th>SCD + G6PD deficiency (n = 7) (age 6–7 yrs)</th>
<th>Mean (SD)</th>
</tr>
</thead>
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<tr>
<td>Hb (g/dL)</td>
<td>6.9 (1.4)</td>
<td></td>
<td>6.6 (2.4)</td>
<td></td>
<td>7.9 (3.2)</td>
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<tr>
<td>PCV (L/L)</td>
<td>21.4 (4.0)</td>
<td></td>
<td>21.2 (7.5)</td>
<td></td>
<td>23.9 (2.5)</td>
<td></td>
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<tr>
<td>RBC (&gt; 10^12/L)</td>
<td>2.31 (0.25)</td>
<td></td>
<td>2.91 (1.31)</td>
<td></td>
<td>2.77 (0.34)</td>
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<tr>
<td>WBC (&gt; 10^9/L)</td>
<td>13.9 (5.2)</td>
<td></td>
<td>14.8 (4.4)</td>
<td></td>
<td>13.1 (4.7)</td>
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<tr>
<td>MCV (fL)</td>
<td>93.3 (7.8)</td>
<td></td>
<td>74.0 (6.9)</td>
<td></td>
<td>874.7 (76)</td>
<td></td>
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<tr>
<td>MCH (pg)</td>
<td>29.9 (3.30)</td>
<td></td>
<td>23.0 (2.4)</td>
<td></td>
<td>28.9 (3.1)</td>
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<tr>
<td>MCHC (g/dL)</td>
<td>32.0 (1.8)</td>
<td></td>
<td>31.0 (1.2)</td>
<td></td>
<td>32.4 (1.7)</td>
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<tr>
<td>Nucleated RBCs (%)</td>
<td>10.3 (15.1)</td>
<td></td>
<td>13.7 (19.4)</td>
<td></td>
<td>73 (10.6)</td>
<td></td>
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<tr>
<td>HbS (%)</td>
<td>86.0 (7.2)</td>
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<td>87.0 (9.3)</td>
<td></td>
<td>85.6 (8.1)</td>
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<tr>
<td>Hbf (%)</td>
<td>9.7 (7.6)</td>
<td></td>
<td>8.5 (9.9)</td>
<td></td>
<td>9.5 (9.1)</td>
<td></td>
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<tr>
<td>HbA2 (%)</td>
<td>4.3 (1.3)</td>
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<td>4.5 (2.0)</td>
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<td>4.9 (1.4)</td>
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<tr>
<td>Serum ferritin (ng/mL)</td>
<td>707 (592)</td>
<td></td>
<td>1174 (919)</td>
<td></td>
<td>611 (615)</td>
<td></td>
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<tr>
<td>G6PD deficiency (mU/10^9 RBC)</td>
<td>127 (16)</td>
<td></td>
<td>90.0 (26)</td>
<td></td>
<td>45.0 (15)</td>
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</tr>
</tbody>
</table>

**Table 3** Relationship of total haemoglobin (Hb) level and white blood cell (WBC) count with fetal haemoglobin (HbF) level

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of samples</th>
<th>HbF (%)</th>
<th>Total Hb (g/dL)</th>
<th>WBC (&gt; 10^9/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>- HbF &lt; 20%</td>
<td>27</td>
<td>71</td>
<td>6.9</td>
<td>14.9</td>
</tr>
<tr>
<td>HbF ≥ 20%</td>
<td>4</td>
<td>27.4</td>
<td>7.9</td>
<td>8.5</td>
</tr>
</tbody>
</table>

*1 case had SCD + thalassaemia + G6PD deficiency.

Hb = haemoglobin; PCV = packed cell volume; RBC = red blood cells; WBC = white blood cells; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration; MCV = mean corpuscular volume; HbS = sickle haemoglobin; Hbf = fetal haemoglobin; HbA2 = haemoglobin alpha 2.

n = number of samples.
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References