A positive correlation between the gene frequency of glucose-6-phosphate dehydrogenase deficiency (G-6-PD), haemoglobin S (αβ6Glu→Val) and malaria endemicity has been demonstrated in different parts of the world. Hb S carriers (Hb AS) and G-6-PD deficient individuals have been shown to have an inborn resistance against *Plasmodium falciparum.*

The presence of Hb S and G-6-PD deficiency genes was first reported in the Saudi population.
during the early 1960s.\textsuperscript{13,14} Thereafter, screening studies in different regions of Saudi Arabia have identified the presence of both Hb S and G-6-PD deficiency genes at variable frequency. Areas that had a past or present history of malaria endemicity have a higher frequency of these genes compared with areas that are non-endemic to malaria.\textsuperscript{15–22}

In this paper we report our findings in six regions of western Saudi Arabia and discuss the reasons for the significant differences in the Hb S and G-6-PD deficiency gene frequencies within malaria endemic regions.

**Materials and Methods**

All subjects investigated in this study were Saudi nationals living in different regions of the Western Province shown in the sketch map (Fig. 1). A total of 6265 blood samples were collected during field trips to each region. Blood samples (5–10 ml) were drawn by venepuncture in EDTA or acid citrate dextrose (ACD) tubes. Fresh blood samples were used to prepare blood smears for red cell morphological studies and estimation of haematological analytes and red cell indices using a Coulter Counter ZF6 with a haemoglobinimeter attachment. The cells, plasma and buffy coat were separated by centrifugation and the red cells were washed twice with cold physiological saline and haemolysed by addition of cold distilled water or 0.02% digitonin. Thereafter, the fresh haemolysate was used for the spectrophotometric estimation of G-6-PD activity using commercially available kits from Boehringer Mannheim (Boehringer Mannheim Diagnostics, GmbH). The activity of G-6-PD (mU/10\textsuperscript{9} erythrocytes per millilitre) was calculated from the change in OD at 340 nm per minute. Phenotypes of G-6-PD were separated by electrophoresis on cellulose acetate plates (Titan III plates, Helena Cat. No. 3023) using Supra Heme buffer (pH 8.6) for 20 min applying 350 V. The bands of G-6-PD were visualized by specific staining (Helena Cat. No. 5620) for 20 min at 20 °C. The plates were fixed in 7.5% trichloroacetic acid for 2–3 min, washed in acetic acid (5%), and air-dried.

The haemoglobin phenotypes were separated by electrophoresis at pH 8.6 on cellulose acetate plates (Titan III plates, Helena Cat. No. 3023)\textsuperscript{23} and confirmed by electrophoresis at pH 6.0 using citrate agar plates and 0.05M sodium citrate buffer.\textsuperscript{24} The haemoglobins A, and F were estimated using an elution technique following alkaline electrophoresis and alkali denaturation,\textsuperscript{25} respectively.

**Results**

Sickle cell haemoglobin (Hb S) was identified both in homozygous and heterozygous states in each region investigated. The Hb S gene frequency was calculated and the results obtained are presented in Table 1. The lowest Hb S gene frequency was encountered in Yanbu and the highest in Al-Qunfuda followed by Jizan. The frequency of severe G-6-PD deficiency (<20% of normal) was calculated in the males and females separately and is presented in Table 1. The G-6-PD phenotypes were identified on the basis of their electrophoretic mobility (Fig. 2) and activity towards glucose-6-phosphate. The

<table>
<thead>
<tr>
<th>Area</th>
<th>Number investigated</th>
<th>Hb S</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jizan</td>
<td>1466</td>
<td>0.103</td>
<td>0.204</td>
<td>0.048</td>
</tr>
<tr>
<td>Al-Qunfuda</td>
<td>823</td>
<td>0.129</td>
<td>0.1106</td>
<td>0.1015</td>
</tr>
<tr>
<td>Makkah</td>
<td>877</td>
<td>0.0274</td>
<td>0.0576</td>
<td>0.0423</td>
</tr>
<tr>
<td>Yanbu</td>
<td>1095</td>
<td>0.0155</td>
<td>0.0179</td>
<td>0.0643</td>
</tr>
<tr>
<td>Bisha</td>
<td>933</td>
<td>0.0831</td>
<td>0.0767</td>
<td>0.054</td>
</tr>
<tr>
<td>Al-Baha</td>
<td>1071</td>
<td>0.0187</td>
<td>0.1275</td>
<td>0.1158</td>
</tr>
</tbody>
</table>

\textsuperscript{a}G-6-PD activity less than 20% of the normal (due to G-6-PD- Mediterranean and G-6-PD-A \textsuperscript{24}).

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Figure 1. Sketch map of Saudi Arabia showing the areas in Western Saudi Arabia screened during this study. The Eastern Province (EP) has a similar topography, high malaria endemicity and a high frequency of Hb S and G-6-PD deficiency. The Northern Province (NP), Southern Province (SP) and Central Province (CP) have significantly lower frequencies of these genes.\textsuperscript{16–18}

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G-6-PD phenotypes included the normal enzymes G-6-PD-B* and a faster moving G-6-PD-A* (an African variant with normal activity), G-6-PD-A* (same mobility as G-6-PD-A* but with activity less than 20% of the normal), G-6-PD Mediterranean (same mobility as G-6-PD-B* but with activity less than 10% of the normal), and G-6-PD-Weak (mobility same as G-6-PD-B* but with activity ranging between 20–60% of the normal).23 The frequencies of each phenotype in males and females are presented in Tables 2 and 3 respectively.

### Table 2

<table>
<thead>
<tr>
<th>Area</th>
<th>No. investigated</th>
<th>G-6-PD-B*</th>
<th>G-6-PD-A*</th>
<th>G-6-PD-A−</th>
<th>G-6-PD-Med</th>
<th>G-6-PD-Weak</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jizan</td>
<td>109</td>
<td>0.602</td>
<td>0.037</td>
<td>0.028</td>
<td>0.176</td>
<td>0.130</td>
</tr>
<tr>
<td>Al-Quinfuda</td>
<td>432</td>
<td>0.836</td>
<td>0.023</td>
<td>0.0046</td>
<td>0.106</td>
<td>0.030</td>
</tr>
<tr>
<td>Makkah</td>
<td>382</td>
<td>0.7172</td>
<td>0.0288</td>
<td>0.0026</td>
<td>0.0550</td>
<td>0.1963</td>
</tr>
<tr>
<td>Yanbu</td>
<td>724</td>
<td>0.953</td>
<td>0.00108</td>
<td>0.00</td>
<td>0.0179</td>
<td>0.0221</td>
</tr>
<tr>
<td>Bisha</td>
<td>469</td>
<td>0.844</td>
<td>0.0043</td>
<td>0.00</td>
<td>0.0767</td>
<td>0.0746</td>
</tr>
<tr>
<td>Al-Baha</td>
<td>519</td>
<td>0.7769</td>
<td>0.0129</td>
<td>0.002</td>
<td>0.1255</td>
<td>0.0817</td>
</tr>
</tbody>
</table>

*G-6-PD-Med: G-6-PD-Mediterranean.
†Uncharacterized variant/s=0.028.

### Table 3

<table>
<thead>
<tr>
<th>Area</th>
<th>No. investigated</th>
<th>G-6-PD-B*</th>
<th>G-6-PD-A*</th>
<th>G-6-PD-A−</th>
<th>G-6-PD-Med</th>
<th>G-6-PD-Weak</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jizan</td>
<td>148</td>
<td>0.797</td>
<td>0.007</td>
<td>0.007</td>
<td>0.041</td>
<td>0.121</td>
</tr>
<tr>
<td>Al-Quinfuda</td>
<td>200</td>
<td>0.765</td>
<td>0.0196</td>
<td>0.0065</td>
<td>0.095</td>
<td>0.095</td>
</tr>
<tr>
<td>Makkah</td>
<td>307</td>
<td>0.6449</td>
<td>0.0033</td>
<td>0.0130</td>
<td>0.0293</td>
<td>0.2606</td>
</tr>
<tr>
<td>Yanbu</td>
<td>315</td>
<td>0.971</td>
<td>0.0095</td>
<td>0.00</td>
<td>0.00635</td>
<td>0.0127</td>
</tr>
<tr>
<td>Bisha</td>
<td>351</td>
<td>0.855</td>
<td>0.0057</td>
<td>0.00</td>
<td>0.054</td>
<td>0.0855</td>
</tr>
<tr>
<td>Al-Baha</td>
<td>328</td>
<td>0.722</td>
<td>0.003</td>
<td>0.003</td>
<td>0.1128</td>
<td>0.1311</td>
</tr>
</tbody>
</table>

*G-6-PD-Med: G-6-PD-Mediterranean.
†Uncharacterized variant/s=0.0017.
‡0.025 double heterozygotes to G-6-PD B*/A− and G-6-PD-B*/G-6-PD-A−.
§0.0489 double heterozygotes to G-6-PD B*/A* (0.0228), G-6-PD-Med/A* (0.0228) and G-6-PD B*/A− (0.0033).
¶0.0273 double heterozygotes to G-6-PD B*/A* (0.0183) and G-6-PD B*/A− (0.0099).
Bisha is also a traditionally agricultural malaria-endemic area with oases planted mainly with palm trees. It has several wadis and villages dispersed along the Sarawat mountain range and has lakes and wells. Both G-6-PD deficiency and the sickle cell gene were encountered and severe G-6-PD deficiency is caused by G-6-PD-Mediterranean. The African variant G-6-PD-A\(^-\) was not encountered, though in all other regions investigated it exists at frequencies ranging from 0.002 to 0.046 in males and 0.003 to 0.013 in the female population.

Al-Baha is different from the other areas investigated in that it is a mountainous area with a much cooler climate and conditions that are less optimal to the growth of the malarial parasite though it has been a malaria endemic region in the past.\(^{26}\) However, both Hb S and G-6-PD deficiency genes were encountered though the frequency of the former is very low (0.0187) and similar to that encountered in Yanbu. The G-6-PD deficiency is due mainly to G-6-PD-Mediterranean and occurs at a high frequency both in males and females.

Jizan, on south-western coast of Saudi Arabia, is similar in topography to Al-Qunfuda and is traditionally an agricultural and malaria-endemic area. Amongst the areas investigated during the study, Jizan had the highest gene frequency of G-6-PD-Mediterranean and follows Al-Qunfuda in regard to the Hb S gene frequency. Unlike the other areas, G-6-PD-A\(^-\) was encountered in both Jizan and Al-Qunfuda at polymorphic levels.

In conclusion, this paper reports and compares the gene frequencies of Hb S and G-6-PD deficiency genes in different regions of Saudi Arabia and shows significant differences between areas that have a past or present history of malaria endemicity. The analysis of microgeographic distribution shows that although the overall distribution of red cell abnormalities in Saudi Arabia support the malaria hypothesis a few exceptions are encountered. It seems that the altitude and climate influence the establishment of the gene frequency of red cell abnormalities to a significant level. There are several similar examples in the literature where exceptions to the ‘malaria hypothesis’ are encountered.\(^{11}\) Yet this hypothesis is considered as the only plausible explanation for the distributions of most of the polymorphisms of red cell defects, due to the natural inborn resistance against malaria which is present in carriers of G-6-PD deficiency and Hb S genes.

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References


