Molecular studies on Yemeni sickle-cell-disease patients: *Xmn* I polymorphism

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SUMMARY Our studies of the Saudi population have shown that in patients with mild presentation of sickle-cell disease (SCD) from Saudi Arabia's eastern region, the prevalence of polymorphic sites is high. However, the prevalence is very low in patients with severe SCD from the south-west of the country. We expanded these studies to a group of Yemeni patients with severe SCD, resident in Riyadh. We investigated a total of 60 chromosomes carrying the sickle-cell (Hb S) gene and 14 chromosomes carrying the Hb A gene. Amongst the Hb AA group, the prevalence was 42.9% and 57.1% for the presence (+) and absence (–) of *Xmn* I polymorphic sites. In the Hb SS individuals, the prevalence of *Xmn* I polymorphic sites was similar to the prevalence reported in the south-western region of Saudi Arabia.

Introduction

Several restriction endonuclease polymorphic sites occur in the β-globin gene cluster on chromosome 11 and some seem to be linked to the clinical expression of sickle-cell disease (SCD) [*1,2*]. In this respect the *Xmn* I polymorphic site 5' to Gγ has received considerable attention [*3,4*]. *Xmn* I normally restricts on both sides of the γ-globin genes producing an 8.1 kb DNA fragment. In some individuals, a cytosine to thymine (C→T) mutation occurs, thus creating a new polymorphic site which results in a shorter 7.0 kb fragment [*3*]. Several studies point to a possible linkage between the presence of this site and mild clinical presentation of SCD, via production of a higher level of Hb F compared with those individuals who do not have the polymorphic site [*3–5*]. In this study we investigated a group of Yemeni SCD patients suffering from severe disease to determine the prevalence of the *Xmn* I polymorphic site, 5' to Gγ-gene.

Patients and methods

The study group comprised 30 Yemeni patients attending King Khalid University Hospital, Riyadh, in whom sickle-cell anaemia had been established on the basis of haemoglobin (Hb) electrophoresis and clinical presentation. During one of their visits for routine follow-up, a 5–10-mL blood sample was drawn by venepuncture in EDTA tubes, and haematological param-

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Received: 20/11/98; accepted: 14/09/99

eters and red cells indices determined using Coulter Counter ZF6 (Beckman Coulter, California, United States of America) with a haemoglobinometer attachment. Blood was also drawn from a group of 7 Hb AA children attending the hospital for minor illnesses. This group was used as controls.

The blood was centrifuged and the plasma buffy coat and red cell layers were carefully separated. The plasma was used for the estimation of biochemical parameters (total protein, albumin, total and direct bilirubin, alanine and asparate transaminase and alkaline phosphatase) on an autoanalyser (American Monitor Parallel). The red cells were washed twice with cold physiological saline and haemolysed with cold distilled water. Fresh haemolysate was used for the confirmation of haemoglobin types on electrophoresis at alkaline [*6*] and acid pH [*7*], and for estimation of Hb AA [*6*] and Hb F [*8*]. The buffy coat layer was used for extraction of DNA by a method published earlier [*9*].

DNA (10 μg) was digested with *Xmn* I following the manufacturer's instructions (New England Biolab, United States of America). The fragments generated were separated by electrophoresis in 0.9% agarose gel for 18–20 hours, transferred to nitrocellulose sheets by Southern blotting [*10*], hybridized to a ^{32}P-labelled probe of γ-globin gene (CHB4 — a gift from Dr Arthur Nienhuis), washed extensively under stringent conditions to remove any unbound probe and dried. The bands were visualized by autoradiography.

Results

The results of haematological parameters in the SCD patients compared with the controls are presented in Table 1 and the biochemical parameters in Table 2. The patients were all suffering from severe anaemia (generally normochromic–normocytic). The white blood cell count was generally higher in all patients. Hb F levels ranged from 2.0% to 15.0%. In all patients except two, Hb A_2 levels were within the normal range. Total and direct bilirubin were significantly elevated in a majority of patients and total protein and albumin levels were slightly lower than normal. Slight elevation of the transaminases was also observed.

The location of the normal and polymorphic site of *Xmn* I and the results obtained in the Yemeni SCD patients and controls are presented in Figure 1. In the 30 Hb SS individuals (60 chromosomes), *Xmn* I digestion produced an 8.1 kb fragment in 29 (96.6%) patients, indicating the absence of an *Xmn* I polymorphic site, while in 1 (3.3%), *Xmn* I digestion produced a 7.0 kb fragment, indicating the presence of an *Xmn* I polymorphic site in homozygous state. This gave a frequency of 96.6% and 3.3% for the absence and presence of *Xmn* I polymorphic sites respectively. Among the 7 Hb AA children, 3 (42.9%) did not have the polymorphic presence of the *Xmn* I site. This gave an overall frequency of 57.1% and 42.9% respectively for the absence and presence of the *Xmn* I polymorphic site.

Discussion

The SCD patients investigated in this study were classified as suffering from a severe form of SCD, as they had frequent episodes of crises often requiring hospitalization, frequently received blood transfusion, and several had fairly low Hb levels. They were on frequent follow-up protocols and attended the hospital regularly. All patients were young (age range: 2–19 years).

Table 1 **Haematological values in Yemeni sickle-cell-disease (SCD) patients and controls**

Parameter	Yemeni SCD patients	Controls
Age (years)	10.00 ± 9.70	11.20 ± 9.10
Red blood cell count (× 10^{12}/L)	3.00 ± 0.40	4.20 ± 0.53
Haemoglobin (g/dL)	9.00 ± 1.60	12.60 ± 1.70
Packed cell volume (L/L)	0.27 ± 0.05	0.39 ± 0.06
Mean corpuscular volume (fL)	87.00 ± 6.00	91.80 ± 14.00
Mean corpuscular haemoglobin (pg)	30.00 ± 5.00	30.00 ± 14.00
Mean corpuscular haemoglobin concentration (g/dL)	33.70 ± 2.70	35.70 ± 3.71
White blood cell count (× 10^{9}/L)	14.00 ± 8.50	4.00 ± 1.30
Hb A_2 (%)	3.50 ± 0.30	2.80 ± 0.55
Hb F (%)	6.50 ± 4.20	1.60 ± 0.58

Values are expressed as mean ± standard deviation.

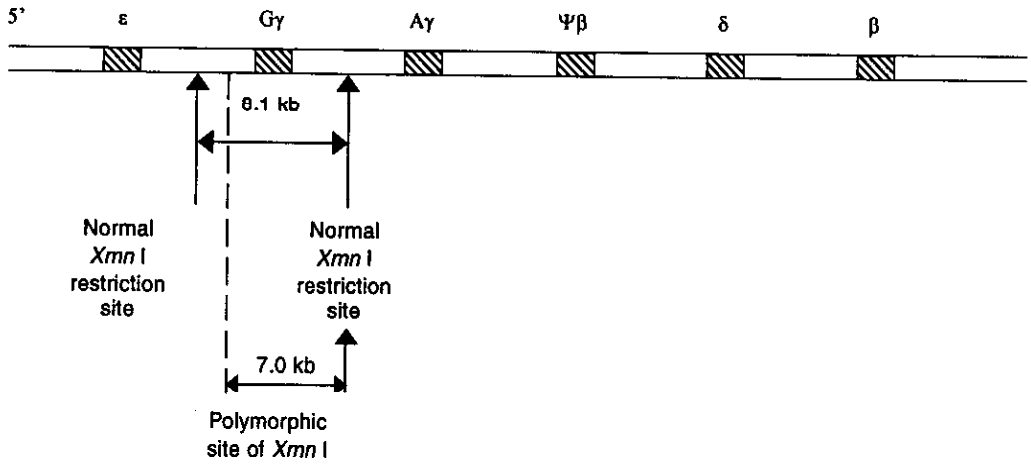
Table 2 **Biochemical values in Yemeni sickle-cell-disease (SCD) patients and controls**

Parameter	Yemeni SCD patients	Controls
Total protein (g/L)	67.0 ± 5.0	74.0 ± 6.0
Albumin (g/L)	40.0 ± 5.2	44.3 ± 4.1
Total bilirubin (μmol/L)	36.5 ± 19.4	9.5 ± 3.8
Direct bilirubin (μmol/L)	10.9 ± 43.5	2.0 ± 1.1
Alanine transaminase (U/L)	25.0 ± 7.5	28.0 ± 9.9
Aspartate transaminase (U/L)	59.0 ± 35.5	25.0 ± 11.0

Values are expressed as mean ± standard deviation.

The haematological parameters showed a fair degree of anaemia, which was generally normochromic–normocytic, and irreversibly sickled cells were seen during the red cell morphological studies. White blood cell counts were elevated. The biochemical parameters also indicated haemolytic anaemia, as total and indirect bilirubin levels were elevated. Some elevation may be due to abnormalities in the liver, since the transaminases were slightly elevated and albumin was reduced.

The γ-globins of Hb are coded by a pair of non-allelic genes located in the β-globin gene cluster on the short arm of chromosome 11. They synthesise different γ-globin chains [*11*] which differ from each only in a single amino acid and are named as Gγ and Aγ [*12–14*]. At different stages of development there is a variable rate of expression of Gγ and Aγ, although the ratio shows significant differences in different populations. Even in diseases such as sickle-cell anaemia, the ratio of Gγ/Aγ shows considerable variation [*15*]. Normally, on both sides of the γ-globin genes there exists a restriction site for *Xmn* I, which produces an 8.1 kb fragment carrying the γ-globin gene upon digestion with *Xmn* I. In some individuals a polymorphic site exists 153 kb 5' to Gγ. In the presence of this site, a smaller 7.0 kb fragment is obtained, as a C→T mutation generates a new restriction site of *Xmn* I. The presence of this site is believed to influence Gγ globin gene expression in patients with sickle-cell anaemia and β-thalassaemia [*3,5*]. Thus, a higher Hb F level is believed to be associated with the presence of the polymorphic site.



Hb phenotype	No. of chromosomes investigated	No. without *Xmn* I polymorphic site (8.1 kb fragment)	No. with *Xmn* I polymorphic site (7.0 kb fragment)	Frequency (%) (–)	(+)
SS	60	58	2	96.7	3.3
AA	14	8	6	57.1	42.9

Figure 1 The *Xmn* I polymorphic site (on the β-globin gene cluster on chromosome 11) and its prevalence in Yemeni sickle-cell-disease patients

In our study, of significant interest was the finding that only one of the SCD patients was homozygous for the 7.0 kb fragment of *Xmn* I (i.e. the polymorphic site was present on both chromosomes). The other 58 chromosomes did not have the *Xmn* I polymorphic site. This gave a very high prevalence (96.7%) for the absence of the *Xmn* I site, with a prevalence of 3.3% for the presence of the polymorphic site. These results are in agreement with those reported from south-western Saudi Arabia, where there was no *Xmn* I polymorphic site in 96.6% of the SCD patients [5], a majority of whom suffered from severe disease. Conversely, in SCD patients in eastern Saudi Arabia, the *Xmn* I polymorphic site was present in 90% of the chromosomes investigated, and the disease was generally much milder, with very low frequencies of crises, hospitalization, blood transfusion and other associated complications.

Additionally, in our studies on Yemenis, we did not observe any close link between the *Xmn* I polymorphic site and Hb F levels. A wide range of Hb F levels (i.e. 2%–20%) was obtained both in the presence and absence of this site. Similarly, in the SCD patients from both the south-western and the eastern regions of Saudi Arabia, Hb F levels ranged from 2% to 22% [5]. From our experience with Saudis, we could not show a link between Hb F levels and SCD severity, although a link seems to exist between disease severity and the presence of the *Xmn* I polymorphic site, where a milder disease is believed to occur in patients with the site. This is also confirmed by

the results of the present study where, in the majority of SCD patients with severe disease, the *Xmn* I polymorphic site was absent. The same influence is seen in Saudi Arabia, where a mild disease is believed to occur in patients from the eastern region with the polymorphic site, but not in patients from the western region with severe disease [5]. More detailed investigations are required to study the exact natural history of SCD and associated states in the Yemeni population.

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