Pattern for α-thalassaemia in Yemeni sickle-cell-disease patients

M.A.F. El-Hazmi [1] and A.S. Warsy [2]

SUMMARY A group of Yemeni patients with sickle-cell disease (SCD) and normal Hb AA individuals living in Riyadh were studied to determine the incidence of the α-gene molecular defect. Blood samples were obtained from 26 SCD patients and 19 controls (the Hb AA group). In the SCD patients the frequency of single α-gene deletion (−α/αα) was 0.346, compared to 0.263 in the Hb AA group. The frequency of two gene deletion (−α/−α) was 0.231 (0.0 for the Hb AA group). In one Hb AA case, a triple α-gene arrangement (ααα/αα) was found (frequency 0.053). The results suggest that α-thalassaemia occurs frequently in Yemeni SCD patients. Further studies to determine the overall frequency of α-thalassaemia in the Republic of Yemen would be of value for patient management.

Introduction

Sickle-cell disease (SCD) occurs in the Republic of Yemen, although the exact gene frequency is unknown [*1*] and information on α-thalassaemia is scarce. The current literature contains no reports on the pattern of interaction of α-thalassaemia and SCD among the Yemeni population.

During our studies in Saudi Arabia, we encountered a number of Yemeni SCD patients attending the Ministry of Health Children's Hospital and King Khalid University Hospital for regular treatment and follow-up, in whom DNA studies showed the presence of associated α-thalassaemia. We conducted a comprehensive molecular analysis to determine the prevalence and nature of α-thalassaemia gene defects in Yemeni patients. Here we report our findings in the Yemeni SCD patients and show a pattern of interaction of Hb S and α-thalassaemia genes.

Patients and methods

The study involved 45 Yemeni children under 14 years attending the Ministry of Health Children's Hospital, and King Khalid University Hospital, Riyadh. Blood samples were collected by venepuncture in EDTA or heparinized tubes during their visits to the hospital. Haematological parameters and red cell indices were estimated in fresh blood using a Coulter Counter ZF6 (Beckman Coulter, California, United States of America) with haemoglobinometer attachment. Reticulocytes were estimated using a previously documented procedure [*2*]. The blood was centrifuged to separate the plasma from the cells and

[1] *Medical Biochemistry Department and WHO Collaborating Centre for Haemoglobinopathies, Thalassaemias and Enzymopathies, College of Medicine and King Khalid University Hospital, Riyadh, Saudi Arabia.*
[2] *Department of Biochemistry, College of Science, King Saud University, Riyadh, Saudi Arabia.*
Received: 26/11/98; accepted: 05/09/99

buffy coat. The plasma was removed and stored frozen at –20 °C. The red cells and buffy coat were carefully separated and the red cells wash-ed twice with cold physiological saline and haemolysed just prior to use by adding cold distilled water. The haemolysate was used to estimate haemoglobin types by electrophoresis at alkaline [3] and acid pH [4], and for determining Hb F [5] and A_2 levels [3].

The plasma was used to estimate biochemical parameters using an autoanalyser (American Monitor Parallel), at the Clinical Biochemistry Laboratory, King Khalid University Hospital. The buffy coat was used for the extraction of DNA according to the procedure previously documented [6]. The DNA was separated and purified, and 10 µg aliquots were used for digestion with restriction endonucleases *Bgl* II or *Bam*HI separately, following the procedure supplied by the manufacturer. The digest was subjected to electrophoresis to separate the DNA fragments on 0.9% agarose gel. The fragments were transferred by Southern blotting to nitrocellulose sheets [7]. The DNA containing the α-globin gene was identified by using a radio-labelled probe of a 1.5 kb fragment of the α-globin gene cloned into the *Pst* I site of PLTN1, and released upon digestion with *Pst* II. The filters were extensively washed and subjected to autoradiography for visualization of bands and determination of band size, by comparison with markers run in the gel along with the samples.

Results

Haemoglobin electrophoresis showed 26 children had the Hb SS phenotype while 19 had Hb AA. The latter group, attending the hospital for minor illnesses, were used as controls. The fragments generated by *Bam*HI and *Bgl* II indicated α-thalassaemia. *Bam*HI produced 14.5 kb, 14.5 kb + 10.5 kb and 10.5 kb fragments in individuals with αα/αα, –α/αα and –α/–α gene arrangements respectively (Figure 1). No individual with– –/ gene arrangement was identified. *Bgl* II produced 12.5 kb + 7.0 kb in αα/αα cases, 15.8 kb + 12.5 kb + 7.0 kb in –α/αα cases and 15.8 kb in –α/–α cases (Figure 2). All gene deletions were rightward deletions. Both one (–α/αα) and two (–α/–α) α-gene deletions were identified in the SCD and Hb AA groups. The frequen-

Table 1 Frequency of α-thalassaemia gene arrangement in Yemeni children with sickle-cell disease (SCD) and controls

Group	α-thalassaemia gene arrangement							
	αα/αα		–α/αα		–α/–α		ααα/αα	
	No.	%	No.	%	No.	%	No.	%
SCD								
(*n* = 26)	11	42.3	9	34.6	6	23.1	0	0
Hb AA controls								
(*n* = 19)	13	68.4	5	26.3	0	0	1	5.3
Total								
(*n* = 45)	24	53.3	14	31.1	6	13.3	1	2.2

Table 2 **Gene frequency of αα/, −α/ and ααα genes in Yemeni SCD patients and Hb AA controls**

Gene frequency	SCD	Hb AA	Total
αα/	0.596*	0.8420*	0.700
−α/	0.404*	0.1316*	0.289
ααα	0.000	0.0263	0.011

**Statistically significant (P < 0.05)*
SCD = sickle-cell disease

cies of α-thalassaemia in the total group, and in the SCD and normal controls separately, are shown in Table 1. One Hb AA child had a triple α-globin arrangement, which produced 12.5 kb, 7.0 kb and 4.0 kb fragments upon digestionwith *Bgl* II, and 17.5 kb with *Bam*HI. The SCD and Hb AA children were grouped on the basis of their α-gene arrangement (i.e. αα/αα, −α/αα or −α/−α) and the prevalence of each gene arrangement was calculated (Table 2) The values of haematological and biochemical parameters were separately calculated in each group, using the *Statistical Analysis System* program at the Computer Centre of King Saud University. The results are presented in Table 3.

Discussion

Both sickle-cell and α-thalassaemia genes occur in Middle Eastern countries with varying frequency [*8,9*]. Some areas, particularly those with a past or present history of malaria endemicity, have a high frequency of both these genes [*8*]; this is seen in one of the Republic of Yemen's neighbours, Saudi Arabia [*9 12*]. As consanguineous marriage and marriage between members of the same tribe are common throughout

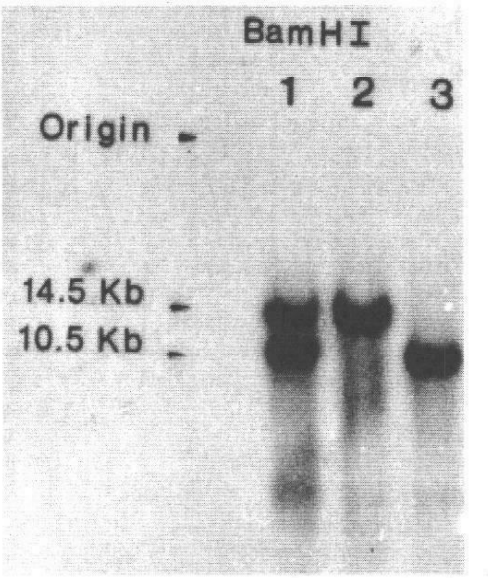

Figure 1 **Autoradiograph of restriction endonucleases *Bam*HI pattern: −α/αα (sample no. 1); αα/αα (sample no. 2); −α/−α (sample no. 3)**

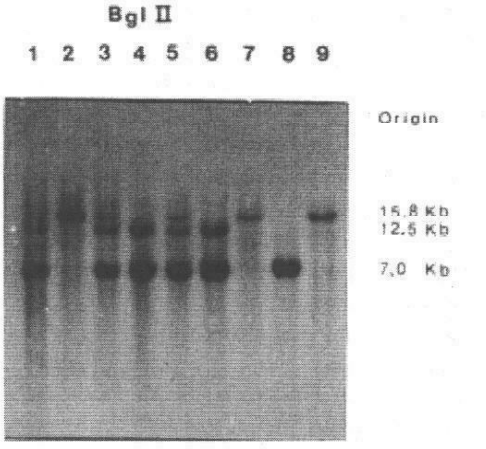

Figure 2 **Autoradiograph of restriction endonuclease *Bgl* II: −α/αα (sample nos 1, 3, 5); −α/−α [sample nos 2, 7, 9, 8 (leftward deletion)]; αα/αα (sample nos 4, 6)**

Table 3 **Haematological parameters in Yemeni SCD patients, with and without α-thalassaemia**

Parameter	−α/−α (*n* = 6)	−α/αα (*n* = 9)	αα/αα (*n* = 11)
Haematological			
Haemoglobin (g/dL)	8.80 ± 0.70	9.00 ± 3.20	9.50 ± 1.60
Red blood cell count ($\times 10^{12}$/L)	3.60 ± 0.77	3.70 ± 3.00[a]	2.80 ± 0.35[c]
White blood cell count ($\times 10^{9}$/L)	11.80 ± 0.70	12.60 ± 5.20	12.30 ± 7.40
Packed cell volume (L/L)	0.25 ± 0.03	0.20 ± 0.04[a]	0.27 ± 0.04
Reticulocytes (%)	6.80 ± 3.80[b]	23.10 ± 16.60[c]	9.80 ± 6.80
Mean corpuscular volume (fL)	73.20 ± 7.50[b]	80.30 ± 6.20[a]	95.40 ± 13.80[c]
Mean corpuscular haemoglobin (pg)	24.80 ± 4.70[b]	31.80 ± 7.80	34.30 ± 6.00[c]
Mean corpuscular haemoglobin concentration (g/dL)	34.30 ± 18.00	35.20 ± 3.60	35.40 ± 1.90
Hb A_2 (%)	4.10 ± 1.50	3.20 ± 0.76	3.30 ± 0.40
Hb F (%)	7.50 ± 3.30	10.10 ± 3.70	5.70 ± 4.60
Biochemical			
Total bilirubin (mmol/L)	28.0 ± 18.0	76.3 ± 38.6	120.0 ± 153.8
Delta bilirubin (mmol/L)	–	10.0 ± 1.4	33.2 ± 41.5
Total protein (g/L)	60.0 ± 20.9	75.3 ± 5.9	70.6 ± 6.2
Albumin (g/L)	30.0 ± 20.0	42.7 ± 8.1	42.6 ± 4.5
Alanine aminotransferase (U/L)	48.0 ± 25.5	43.0 ± 10.0	23.0 ± 1.4
Aspartate aminotransferase (U/L)	79.0 ± 5.6[b]	15.5 ± 6.4	58.2 ± 36.9
Alkaline phosphatase (U/L)	163.0 ± 4.2	275.3 ± 87.0	167.4 ± 41.8
Lactate dehydrogenase (U/L)	269.5 ± 98.3	783.7 ± 197.6[a]	278.3 ± 188.6
Ferritin (ng/mL)	276.2 ± 208.0	190.6 ± 159.2	355.8 ± 264.0

[a] *Statistically significant (P < 0.05) compared to SCD with αα/αα*
[b] *Statistically significant (P < 0.05) compared to SCD with −α/αα*
[c] *Statistically significant (P< 0.05)* compared to SCD with *−α/−α*
Values are presented as mean ± standard deviation.

the region [*13*], coexisting α-thalassaemia and SCD cases occur, influencing the presentation of SCD [*14*]. Because of Saudi Arabia's close proximity to the Republic of Yemen (we have reported a high frequency of Hb S and α-thalassaemia genes in the south-western region of Saudi Arabia [*8,9–12*]), we expected to find a high frequency of these genes in the Republic of Yemen. This expectation was confirmed by the findings of Haider [*1*].

Our study showed a number of interesting findings. First, in the group overall, 31.1% were found to have one α-gene (−α/αα) deletion, and 13.3%, two α-gene deletions (−α/−α), giving a gene frequency for −α/ of 0.289. Secondly, the frequency of α-thalassaemia was significantly higher (0.404) in the children with SCD (−α/αα = 34.6%; −α/−α = 23.1%) compared with the children with Hb AA (0.1316), where 26.3% had one α-gene deletion and none had two α-gene deletions. One case of a triple α-gene arrangement was seen, giving an ααα/− frequency of 0.011.

The α-gene deletion is a rightward deletion which results in the deletion of a 3.7 kb fragment comprised partly of α_1 and α_2

globin genes, thus producing a hybrid [*15,16*]. Cases of leftward deletion are not seen in Saudi Arabia, although α-thalassaemia due to the occurrence of one or two α-gene deletions is frequent. The leftward deletion has been identified, but at a very low frequency [*17,18*]. Instances of triple α-gene arrangement have been reported in Saudi Arabian Hb AA and SCD cases, also at a very low frequency [*19,20*].

Within the SCD group, when the children without α-thalassaemia (αα/αα) were compared to those with one (−α/αα) or two (−α/−α) gene deletions, some statistical differences were seen in the haematological parameters. The red cell count was highest in the −α/αα and lowest in the αα/αα groups, while packed cell volume was lowest in the −α/αα group. Red cell indices decreased significantly as the number of α-genes deleted increased. Reticulocytes were higher in SCD with one α-gene deletion, but significantly lower in those with two α-gene deletions. This unexpected result may be due to a number of children with very high rates of haemolysis and hence reticulocytosis.

Among the biochemical parameters, several differences were seen but none was statistically significant. These results match results from Saudi Arabia for some parameters, but not all [*14*].

This preliminary report on SCD in Yemeni children shows a higher frequency of α-thalassaemia in the SCD group compared with that of the Hb AA group. Whether the associated α-thalassaemia has any ameliorating effect on the nature of SCD in Yemenis is yet to be determined.

References

1. Haider NAA. Sickle-cell disease Hajjaj, Yemen Republic (Abstract). In: *Symposium on diseases in the Arab population. A wealth of indicative information, 23–24 November, 1997.* Riyadh, Saudi Arabia, College of Medicine, King Saud University.
2. Dacie JV, Lewis SM. *Practical haematology.* 5th ed. London, Churchill Livingstone, 1975.
3. Marengo-Rowe AJ. Rapid electrophoresis and quantitation of haemoglobins on cellulose acetate. *Journal of clinical pathology*, 1965, 18:790–2.
4. Robinson AR et al. A new technique for differentiation of hemoglobin. *Journal of laboratory and clinical medicine*, 1958, 50:745–52.
5. Betke K et al. Estimation of small percentage of foetal haemoglobin. *Nature*, 1959, 84:1877–8.
6. Kunkel LM et al. Analysis of human Y-chromosome-specific reiterated DNA in chromosome variants. *Proceedings of the National Academy of Sciences of the United States of America*, 1977, 74:1245–9.
7. Southern EM. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *Journal of molecular biology*, 1975, 98:503–17.
8. El-Hazmi MAF, Warsy AS. Hemoglobinopathies in Arab countries. In Teebi AS, Faraj J, eds. *Genetic diseases among Arab population.* New York, Oxford University Press, 1997:83–110.
9. El-Hazmi MAF. Haemoglobin disorders: a pattern for thalaeeaomia and haomoglobinopathies in Arabia. *Acta haematologica*, 1982, 68:43–51.

10. El-Hazmi MAF. Haemoglobinopathies, thalassaemias and enzymopathies in Saudi Arabia: the present status. *Acta haematologica*, 1987, 78:130–4.

11. El-Hazmi MAF. The distribution and nature of hemoglobinopathies in Arabia. In: Winter WP, ed. *Hemoglobin variants in human populations*. Boca Raton, Florida, CRC Press, 1987:65–77.

12. El-Hazmi MAF. Abnormal hemoglobins and allied disorders in the Middle East, Saudi Arabia. In: Bowman J, ed. *Distribution and evolution of hemoglobin and globin loci*. New York, Elsevier, 1983:239–49.

13. El-Hazmi MAF et al. Consanguinity among the Saudi Arabian population. *Journal of medical genetics*, 1995, 32:623–6.

14. El-Hazmi MAF. Clinical manifestation and laboratory findings of sickle-cell anaemia in association with alpha-thalassaemia in Saudi Arabia. *Acta haematologica*, 1985, 74:155–60.

15. Liebhaber SA et al. Homology and concerted evolution at the alpha 1 and alpha 2 loci of human alpha-globin. *Nature*, 1981, 290:26–9.

16. Maniatis T et al. The molecular genetics of human hemoglobins. *Annual review of genetics*, 1980, 14:145–78.

17. El-Hazmi MAF. Alpha-thalassemia in Saudi Arabia: deletion pattern. *Human genetics*, 1987, 76:196–8.

18. El-Hazmi MAF. Leftward deletion α-thalassemia in the Saudi Arabian population. *Human genetics*, 1986, 74:219–22.

19. El-Hazmi MAF. Haplotypes of α-globin-gene in the Saudi population — the triplicated gene (alpha alpha alpha anti$^{3.7}$). *Human heredity*, 1987, 37:314–8.

20. El-Hazmi MAF et al. Triple α genes in association with sickle-cell and β-thalassaemia gene in the Saudi population. *Acta haematologica*, 1987, 77:161–5.