Toward prevention of Hemoglobinopathies in Oman

Hassan, S.M.

CHAPTER 9

HAPLOTYPES, SUB-HAPLOTYPES AND GEOGRAPHICAL DISTRIBUTION IN OMANI PATIENTS WITH SICKLE CELL DISEASE

Hassan SM, Al Muslahi M, Al Riyami M, Al Balushi A, Bakker E, Harteveld CL and Giordano PC

Thalassemia Reports. 2015; 5(4739): 6-11

ABSTRACT

Introduction

Despite the fact that patients homozygous for the sickle cell disease (SCD) mutation have an identical genotype, the severity of the disease can be extremely variable. The HbS mutation has been described on five different haplotypes with different clinical expression. Identifying the genotypes, haplotypes and sub-haplotypes of the β gene cluster in Oman needs to be studied in more details to establish a correlation between the genotype/haplotype and phenotype diversity observed in SCD patients for prognostic purposes, accurate diagnosis and thus planning for the best tailored treatment.

Methods

We have investigated 125 HbS homozygotes from different parts of Oman and determined their haplotypes and sub-haplotypes and correlated this to the hematological and clinical expression.

Results

We have found 11 haplotype combinations differently distributed in the country. The Asian/Asian HbS haplotype was the most predominant (37.6%) and was associated with a milder disease. The Benin/Benin came second (20.0%) and was associated with a more severe condition. A new haplotype, in combination with Asian, which we called Asian/OmanI was the third most common (11.2%), CAR/CAR (10.4%) and CAR/OmanI were fourth (10.4%) and CAR/Asian fifth (6.4%). Other haplotype combinations were found at a lower frequency (4%). In patients with CAR/OmanI haplotype, 3 different sub-haplotypes were found. As expected, the correlation between haplotypes, sub-haplotypes and disease severity was mainly associated with HbF expression.

Conclusion

Our study on haplotype/phenotype correlation has shown which major haplotypes occur in the different regions of Oman. Furthermore, neither the haplotype or sub-haplotype nor the HbF alone appeared to be fully associable with the variable clinical phenotypes. External factors do occur and are associated with the expression of the disease.

INTRODUCTION

Sickle cell disease (SCD) is one of the most common autosomal recessive disorder in human and was first described by Herrick in 1910 (1). The disease is caused by a single nucleotide transversion at codon 6 GAG>GTG (HBB:c.20A>T) (NM_000518.4) of the beta globin gene resulting into the commonest haemoglobin variant worldwide (HbS) characterized by the single Glu®Val amino acid substitution at position β6 (2). Despite the fact that all patients homozygous for the HbS allele have an identical genotype, the severity of the disease can be extremely variable among affected subjects (3). The disease may manifest with full blow severity, with chronic and acute infarctions in organs and tissues causing excruciating pain episodes (crisis), brain infarctions, splenic infarction, massive hemolytic events and acute chest syndrome with risk of premature death. Other cases however, may present with milder symptoms and the variability is mainly associated with the haplotype, sub-haplotype, alpha thalassemia (4) and the presence of fetal hemoglobin (HbF) ($\alpha 2\gamma 2$) (8) which may be attributed to the coinheritance of Xmn-I polymorphism. This marker is important for early prevention or reduction of morbidity in SCD patients treated with hydroxyurea therapy (10) or to decide whether or not bone marrow transplantation should be considered (11).

The HbS mutation has been described on five distinct haplotypes based on the presence or absence of the 5 different restriction enzyme sites in the beta-globin gene cluster located on the 5' and two restriction enzyme sites on the 3' sides of the beta gene. These haplotypes are known as Benin, Bantu or Central African Republic (CAR), Senegal, Cameroon and Asian (5). The first four are African haplotypes, named after their origin and ethnic group (6) while the last was described in Central India and Saudi Arabia (7). It has been previously reported that the CAR haplotype is usually associated with a more severe disease when compared with the intermediate phenotype of the Benin haplotype and to the milder conditions associated with the Senegal and Asian haplotypes (9). Therefore, analysis of the polymorphic sites of the β genes cluster is of genetic, anthropologic and clinical interest, and it can also be used to predict the prognosis of the disease and to plan a tailored treatment.

The work reported here involves the investigation of a serial of polymorphic sites (SNP's) within the beta globin gene cluster to identify the HbS haplotype of Omani patients. For this, we have selected 125 Omani SCD patients with homozygous HbS conditions and compared their haplotype with haematological and clinical data. Moreover, to look if there are any sub-haplotypes within each known haplotype that might be associated with the clinical differences seen in patients with the same HbS haplotype, extra SNP's, in addition to the common ones have been studied. Looking for correlations with the clinical phenotypes, we have also charted the distribution of the beta gene haplotypes in different regions of the country and characterized the different haplotypes and sub-haplotypes using advanced molecular technologies

MATERIALS & METHODS

Subjects

We have collected, with signed consent of patients and families, EDTA blood samples from a cohort of 125 SCD patients, whether admitted or following up in one of the Ministry of Health Hospitals in Oman. Gender distribution was; 84 males (45%) and 103 females (55%). The age of the subjects was on average 36 years.

Strategy

Cation-exchange high performance liquid chromatography (HPLC) was performed on all samples on either D-10 (short and extended programs) device and/or Variant II (Bio-Rad Laboratories, Hercules, CA, USA) to measure the rate of HbF/HbS (12) in absence or before blood transfusion. DNA was extracted using the Qiagen kit according to the manufacturer instruction as previously described (13). 47 SNP's, covering 13 sites in the β-globin gene cluster that are known to be variable and informative were analysed (Figure 9.1). This was carried out by an asymmetric PCR (31) and analysed by melting curve analysis (MCA) (14). The common haplotyping of the β-globin gene cluster was determined according to the presence (+) or absence (-) of a composition of single nucleotide polymorphisms (SNP's) corresponding to the traditional five 5' known polymorphic restriction endonuclease sites (F1, F2 (SNP 2), F3 (SNP 2), F4 and F5) (31). The remaining 42 SNP's were used to look for sub-haplotypes to find out if there are nucleotide variations within similar haplotypes that might be associated with the phenotypical differences observed. F1 contains 1 SNP in the 5' region of the ε-gene. The preGframe in the 5' region of the Gγ-gene contains 5 SNPs that are linked and only occur in a limited number of combinations. The promoter regions of the γ-genes; promGγ and promAγ contain 10 SNPs each, among which are the non-deletional HPFH point mutations. Also among the Gγ promoter is the Xmn-I site which is known to cause continued expression of HbF during adult life in case of erythropoietic stress. F2, in intron 2 of the Gγ-gene, and F3, in intron 2 of the Aγ-gene both contain 4 SNPs. F4 contains 1 SNP in the pseudo β-gene. F5 contains 1 SNP in the 3' region of the pseudo β-gene. F6 contains 1 SNP in the 3' region of the δ-gene. The voBRsa1 fragment in the 5' region of the β-gene contains 5 SNPs. This fragment also contains a very polymorphic $(AT)_xT_y$ repeat located after SNP 1 that includes SNP 2. The β-frame in the β-gene contains 3 linked SNPs that only occur in a limited number of combinations. The naBHpa1 fragment in the 3' region of the β-gene contains 1 SNP. F7 in the 3' region of the β-gene contains 1 SNP.

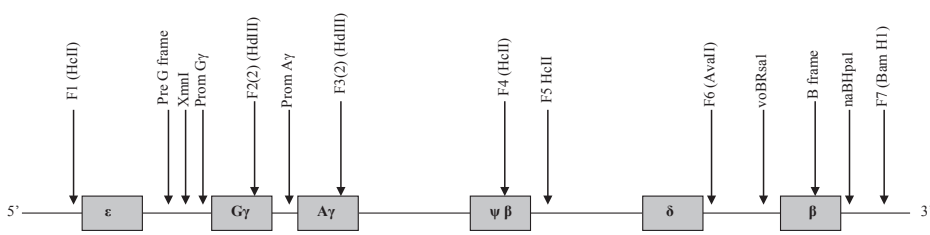

Figure 9.1. Schematic representation of the β-globin gene cluster. The arrows indicate the locations of the 13 different regions that contain the 47 SNPs, including the 5 sites screened for the traditional haplotypes. In addition, the XmnI site has also been indicated in the figure.

Genotyping procedures

Genotypes of PCR products were determined using the Light Scanner, (Idaho Technologies Inc. USA) after performing asymmetric PCR with LCGreen fluorescent DNA dye and unlabelled

oligonucleotide probes (31). The PCR was performed asymmetrically so that the strand complementary to the probe is produced in excess, allowing probe annealing at the SNP site. The primers were designed to yield a product not larger than 200bp as it is the optimal length for accurate scanning and genotyping with MCA. A fluorescent dye (LC Green plus) that emits light in the presence of double stranded DNA was added to the reaction and as the temperature increases, the fluorescence decreases as the dsDNA melts out; this produces a characteristic melting curve (15). Unlabelled oligonucleotide probes were designed and used to genotype targeted sequence variations. These probes increases specificity of the melting reaction as it decreases the size of the product that is melted. Therefore, the probe is designed to anneal to either the wild type or the mutant allele; the characteristic melting curves identify the genotype of each sample (15).

The shape of the PCR amplicon melting curve reveals the presence or absence of the SNP in comparison to the wild type sequence, allowing clear recognition and genotyping (14). The haplotype/sub-haplotype is then drawn from the obtained genotypes. In case of homozygosity for all markers, the haplotype can be defined. In the case of a single SNP difference, two distinct haplotypes can be defined. In case of two or more SNP differences, the most likely/frequent haplotype in the population is defined. SNP's that were doubtful were sequenced by Sanger sequencing for confirmation.

Phenotype Classification

In order to define disease severity, a number of well-defined clinical parameters were analyzed: hemoglobin level, frequency of transfusion which is based on episodes of acute hemolysis (e.g pulmonary hypertension, jaundice, gallstones, splenic crisis), number of annual hospitalization, frequency of painful crises, splenectomy (indicated by acute splenic sequestration and chronic hypersplenism), acute chest syndrome (ACS), body pain (e.g abdomen, chest, bones, joints, episode of dactylitis) and records of any major organ damage such as heart and liver (Table 9.1).

The severity of disease expression was then correlated with the haplotype and hematological parameter readings of HbF and HbS.

Table 9.1. Classification criteria for the 125 homozygous HbS/S patients to assess SCD severity into mild, intermediate and severe.

Clinical classification of SCD patients (n = 125)	Mild n = 46	Intermediate n = 31	Severe n = 48
Haemoglobin level	↑ 9.1	↓ 8.5	↓ 8
Transfusions/haemapheresis	Not required	Occasional	Frequent
Hospitalizations per year	1 - 2	2 - 4	> 5
Crisis frequency	↓ 3 per year	↑ 3 per year	↑ 6 per year
Splenectomy	Yes	Yes	Yes
Acute Chest Syndrome (ACS)	No	No	Yes
Body Pain	mild	moderate	intense
Severe organ damage	No	No	Yes

RESULTS

Determination of Genotypes and Haplotypes

A total of 125 selected patients with SCD that were found to be homozygous for HbS were studied for their β-gene cluster haplotype. The 250 chromosomes from the 125 patients with identical homozygous HbS/S genotype showed 11 different haplotype combinations that were defined by melting curve analysis. The homozygous Asian haplotype was the most predominant (37.6%). The second most prevalent was the homozygous Benin haplotype (20.0%). The remaining haplotypes were distributed as follow: compound heterozygous Asian/OmanI (11.2%), homozygous CAR (10.4%), compound heterozygous CAR/OmanI (10.4%), CAR/Asian (6.4%), homozygous OmanI (0.8%), compound heterozygous of Senegal/OmanI (0.8%), Benin/OmanII (0.8%), Benin/OmanIII (0.8%) and finally Asian/OmanIV (0.8%). Data are summarized in Table 9.2.

Clinical severity

The phenotypes of the patients were classified into mild, intermediate and severe based on the described criteria (Table 9.1). Data were obtained from patient's medical records anonymously provided by the doctor. Among the Asian/Asian haplotype, mostly were presented with a mild disease and none with a severe form. Conversely, Benin/Benin ranged from severe to intermediate while none presented with the mild form. Among the Asian/OmanI haplotype, the percentage was equal between mild and intermediate. The majority of homozygous CAR and compound heterozygous CAR/OmanI beta cluster had a severe clinical profile. Finally, the phenotype of Asian/CAR haplotype ranged from mild to intermediate and even severe cases. Data are summarized in Table 9.2.

Hematological data

The Hb F value correlates with the different haplotypes, giving a direct indication of the disease severity. The average hemoglobin values, HbF and HbS percentage of each haplotype are summarized in Table 9.3. The Asian haplotype, being associated with a mild phenotype, presented with the highest expression of HbF% and the lowest HbS% when compared to the other haplotypes. Patients with Asian/OmanI haplotype had a mild to intermediate phenotype. This can be attributed to the elevated expression of HbF% (Table 3). Conversely, the homozygous CAR and compound heterozygous CAR/OmanI haplotypes had the most severe clinical picture with the lowest Hb F values (Table 3).

Table 9.3. Summary of hematological data (average) for each haplotype

	Asian/Asian	Benin/Benin	Asian/OmanI	CAR/CAR	CAR/OmanI	CAR/Asian
Hb g/dl	9.5 ±1.3	9.3 ±1.7	10.3 ±1.9	7.5 ±1.1	8.7 ±1.2	9.1 ±1.4
HbF %	18.2% ±5.9	6% ±3.1	14.2% ±5.5	5.9% ±4.9	5% ±3.5	12.6% ±5.4
HbS %	72.3% ±6.9	80.3% ±5.7	75.2% ±7.6	80.7% ±7.7	78.5% ±9.1	78.3% ±7.9

Table 9.2. Summary of the genotypes from which the 11 haplotypes were determined in the Omani HbS/S homozygous patients using the five 5′ traditional sites. The Xmn-I genotype and percentages of mild, intermediate and severe cases within each haplotype is also indicated.

	HcII (5′ ε)	Xmn-I	Hd III (Gγ)	Hd III (Aγ)	Hc II (ψβ)	Hc II (3′ψβ)	Ava II (β)	Bam HI (3′β)				
Haplotype	F1 (A: +/C: -)	(C: -/T: +)	F2(2) (G: - /T: +)	F3(2) (G: - / T: +)	F4 (G: -/ A: +)	F5 (G:- / A: +)	F6 (G: -/C:+)	F7 (C,T:-/ A:+)	Total	Mild%	Intermediate%	Severe%
Asian/Asian	AA	TT	TT	GG	AA	AA	GG	C,T/C,T	47	78,7	21,3	0
Benin/Benin	CC	CC	GG	GG	GG	AA	GG	AA	25	0	36	64
Asian/Oman I	AC	TC	TT	GG	GA	AA	GG	A/C,T	14	50	50	0
CAR/CAR	CC	CC	TT	GG	GG	GG	GG	AA	13	0	7,7	92,3
CAR/Oman I	CC	CC	TT	GG	GG	GA	GG	AA	13	0	25	75
CAR/Asian	AC	TC	TT	GG	GA	GA	GG	A/C,T	8	12,5	37,5	50
Oman I/Oman I	CC	CC	TT	GG	GG	AA	GG	AA	1	0	0	100
Senegal/OmanI	CC	TC	TT	GG	GA	AA	GG	AA	1	0	0	100
Asian/Oman II	AA	TC	TG	GG	GA	GA	CG	C,T/C,T	1	0	0	100
Benin/Oman III	AC	CC	TG	GG	GG	GA	CG	AA	1	0	0	100
Benin/Oman IV	CC	CC	GG	GG	GG	GA	GG	AA	1	100	0	0

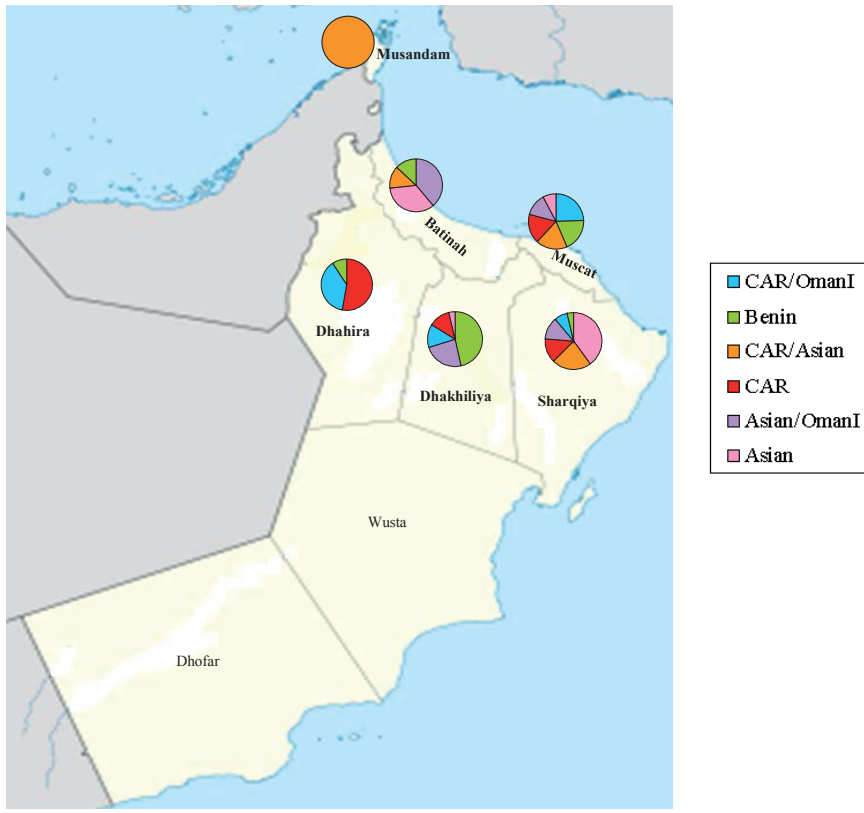

Figure 9.2. The geographical distribution of the β globin gene cluster haplotypes within Oman.

Geographical distribution

The overall distribution of each haplotype in our SCD patients in the different regions of Oman is presented in percentages in Table 9.4. The occurrence frequency of each haplotype in a particular region is depicted in Figure 9.2. In Musandam, CAR/Asian is the only haplotype found. In Batinah, Asian/OmanI is the most abundant. In Muscat, CAR/OmanI is found at the highest frequency and in Sharqiya, the Asian haplotype was the most prevalent. In Dhakhiliya, Benin was the major haplotype found and finally in Dhahira, the CAR haplotype was the most prominent. Data are summarized in Figure 9.2.

Sub-haplotypes

Based on additional SNP's, a subdivision could be made from the original five HbS haplotypes. Trying to find a molecular explanation for the different phenotypes seen within similar basic haplotypes, sub-haplotypes were determined by looking at a total of 42 SNP's in all the125 homozygous HbS patients. Out of the 42 SNP's, only 15 SNP's were found modifying the 11 identified haplotypes. However, no sub-haplotypes were found to be associated with a specific haplotype except for the CAR/OmanI that showed nucleotide variations at the G-γ (SNP1) (SNP position: 5232979-5232984)

Table 9.4. The frequency distribution of the 6 most common haplotypes in 6 different regions in Oman.

Region\ Haplotype	Asian/Asian	Benin/Benin	Asian/OmanI	CAR/CAR	CAR/OmanI	CAR/Asian
Musandam	–	–	–	–	–	12,50%
Batina	31,90%	12%	35,70%	–	–	12,50%
Muscat	21,30%	52%	35,70%	46,20%	66,70%	50%
Sharqiya	44,70%	4%	14,30%	15,30%	8,30%	25%
Dhakhiliya	2,10%	28%	14,30%	7,70%	8,30%	–
Dhahira	–	4%	–	23,10%	16,70%	–
Total %	100	100	100	100	100	100

located in the G-gamma promoter region from which three different sub-haplotypes were defined (a,b and c) (Table 9.5). This site might be associated with the different clinical expression observed in CAR/OmanI patients. The patient with sub haplotype (CAR/OmanI - c) presented with a very severe clinical manifestations and no improvement was seen even after doubling the dosage of hydroxyurea. This patient appears to be homozygous for the wild type 6 nucleotides (CTTTAA) at the G-gamma promoter. On the other hand, another patient with sub haplotype (CAR/OmanI - b) was homozygous for the deletional mutation of the 6 nucleotides at the same position and had a milder clinical presentation and was not taking hydroxyurea consistently as the patient was feeling better after a short period of being on the drug. The remaining patients with sub-haplotypes (CAR/OmanI – a) had the compound heterozygous composition of CTTAA/6nt del at the G-gamma promoter region, needed higher than the average dose of hydroxyurea in order to observe a reduction of the severe phenotype. Although more case studies are needed to confirm our hypothesis, our data allow us to assume that carrying the 6 nucleotide deletion might be beneficial to CAR/OmanI patients for better response to hydroxyurea.

Table 9.5. The different genotypes observed at the G-gamma promoter (SNP1) among CAR/OmanI patients, suggesting the existence of three different sub-haplotypes (a,b and c).

CAR/OmanI sub-haplotype	Gγ(1)	No. of patients	Response to HU
a	CTTTAA/6nt del	11	Mild
b	6nt del/6nt del	1	Positive
c	CTTTAA/CTTTAA	1	None

Hydroxyurea (preliminary data)

The clinical symptoms in severely affected patients that were put on hydroxyurea therapy improved in general but at different levels. As mentioned above, only in patients with CAR/OmanI haplotype, the drug dosage had to be doubled to see some improvements in the treated subjects. However, the different effect of hydroxyurea therapy in correlation with the haplotypes requires further analysis and results will be presented in another study.

DISCUSSION

Haplotypes distribution and disease severity

The Omani populations are known for their high incidence of hemoglobinopathies, including alpha and beta-thalassemia as well as sickle cell disease (13). As mentioned above, patients with SCD present with a variable clinical picture ranging from severe to very mild forms, where haplotypes have been found to be associated with the severity of the disease (20, 21). In Daar et al, a study conducted in Oman in 2000 on 52 HbS/S individuals, it was found that the Benin/Benin haplotype was the most prevalent and twice more frequent than the Asian/Asian (16). In the present study however, the Asian/Asian haplotype was the most prominent while Benin/Benin was the second in rank (Table 9.2). The reason for the higher percentages of Benin haplotype in Daar et al, might be due to a selection among patients attending the Sultan Qaboos University Hospital which are mainly from the Dhakhiliya region and based on our findings, the Benin haplotype has been observed to be present at a high rate in this region (Figure 9.2). Although the effect of these haplotypes on the phenotypes is clearly correlating with the HbF expression, disease severity remains variable within the same haplotypes and more molecular and external factors need to be taken into consideration. The high frequency of alpha-thalassemia reported in the Omani population (13) is another modulating factor influencing the clinical outcome of the disease. The effect of alpha thalassemia on the clinical expression of SCD is under evaluation in our cohort and will be presented in another paper.

Gene flow

The distribution of the HbS haplotypes in Oman is explained by the historical migrations from Zanzibar and India. The presence of the Asian haplotype can be attributed to ancient migrations and to centuries of trade with India and Pakistan. Contacts with East Africa, Zanzibar and Mombasa, explain the presence of the Benin and CAR haplotypes (16). Muscat, being the capital, had the widest diversity of different haplotypes. This reflects more recent migrations of "native people" from the interior to the capital seeking for jobs and better lives. The Asian haplotype was highest in Sharqiya and Batinah and these regions are known to have SCD patients with a mild clinical profile in comparison to Dhakhiliya and Dhahira regions in which they have a more severe manifestation of the disease and this could be explained by the Benin and CAR haplotypes in these regions respectively (Figure 9.2). Only one haplotype combination was found among patients from Musandam (CAR/Asian) and this can be due to the isolation of this region from the rest of the country by mountains and the UAE. SCD is absent in Wusta and Dhofar due to low levels of malaria in the past in these two regions (17). The 4 identified haplotypes which we have referred to as OmanI, II, III and IV are expected in an admixed population such as Oman and might have been derived differently by recombinant events (32). OmanI could have probably been derived from CAR with a mutation at F5. It is also possible to say that it is a result from a recombination event between CAR and Cameroon haplotypes but this is less likely as Cameroon haplotype was not found in the Omani population. Oman II could be a result of a recombinant event between Benin and CAR while OmanIII could be the outcome of a recombination between Asian and CAR haplotypes. Oman IV could be a derivative of OmanII with a mutation at F1. However, these are just assumptions and further studies are eventually required to track back the origin of each haplotype.

Early diagnosis, haplotype, HbF and prognosis

Identifying the disease at an early stage and defining genotype and haplotype allows clinicians to predict to some extent the prognosis and to plan a tailored treatment. Early prediction of the clinical expression will help in preventing or reducing acute painful episodes (crisis) in this cohort, which is the most common traumatic experience in SCD (18), and acute chest syndrome (ACS) which is the most common cause of death in Omani SCD patients (19).

The Asian haplotype was associated with highest HbF levels, fewer hospitalizations and painful episodes and patients did not develop acute chest syndrome although vaso-occlusive events did occur. Our study also confirms that the CAR haplotype whether homozygous or combined heterozygous is associated with lowest HbF level and the highest incidence of organ damage and renal failure as reported elsewhere (22, 23).

Carriers of the HbS gene on the Asian haplotype on one chromosome and OmanI haplotype on the other presented in our cohort high HbF levels (average 14.2%) and a milder clinical course than other compound heterozygous haplotypes. Bakioglu al. (25) reported mild SCD cases of Asian/Benin haplotype with high levels of Hb F (average 22.2%). This shows that carrying the HbS mutation on an Asian haplotype on one chromosome could still contribute to elevating the HbF expression.

Higher concentrations of HbF in the cell lead to lower concentrations of HbS (24), better oxygenation and less clinical severity. A potential threshold of 20% HbF has been suggested to effectively prevent recurrent vasoocclusive episodes (26). This is true in most cases, however, in another study, some patients with HbF levels near 20% had a devastating disease manifestation (18). The same observation was seen in one of the patients in our cohort, with the Asian/CAR haplotype. Despite the high HbF (19.7%), the patient is frequently admitted to the hospital with vaso-occlusive crisis, dactilitis and severe abdominal pain. This patient has no iron overload and is heterozygous for the α-3.7 deletion. This finding imply that other circumstantial or genetic factors or external transacting determinants such as blood viscosity, elevated PCV, vascular adherence, acidosis and dehydration as well as patient's life style and diet might contend with the beneficial effect of the high HbF level. Our findings support the conclusion by Acquaye et al. (27) and Seltzer et al. (28) that fetal hemoglobin levels are very important but not the only parameters that mitigate the severity of the disease and we are at the moment inquiring which other factors could be associated with the severity of this case.

Sub-haplotype

The sub-haplotype study of 42 SNP's on the 11 haplotype combinations observed in our 125 SCD patients revealed that 15 different positions differentiate the 11 identified haplotypes. No sub-haplotypes were determined except in patients with the CAR/OmanI haplotype, which revealed variation at the G-gamma promoter SNP-1 giving 3 different sub-haplotypes (Table 9.5; a, b and c). One patient with sub-haplotype-c who presented with homozygosity for the wild type 6 nucleotide sequence (CTTTAA) at the G-gamma promoter, a very severe phenotype and not responding to high doses of Hyrdoxyurea treatment. This element could be the cause of low HbF expression and of non-response to Hydroxyurea and this hypothesis seems to be sustained by the fact that another patient with sub-haplotype-b with a homozygous mutational

deletion of the 6 nucleotides at the same position became better as soon as being on the drug. The presence or absence of the 6nt sequence in at the G-gamma promoter in the CAR/OmanI patients could be associated with the differences in clinical presentation and with response to hydroxyurea therapy, probably an element is in linkage to the 6nt deletion and is responsible for the positive response to the drug.

CONCLUSION

Our study on haplotype/phenotype correlation has shown the existence of at least 11 different haplotype combinations in Oman. These are differently distributed among the six main regions of the country. Sub-haplotype was only observed in CAR/OmanI combination and could be associated with the clinical differences observed in patients with the same haplotype. Identifying haplotypes and sub-haplotypes in early life may allow a better prognosis and a more accurate risk predictions and a better tailored therapy, to match disease-related risks and to facilitate planning of clinical trials to prevent the development of severe complications later in life. Nevertheless, we have shown that when the phenotypes are classified into; mild, intermediate and severe, neither the haplotype or the HbF alone appeared to be fully associable with the clinical phenotypes as also been observed by Alexander et al (29). External and/or modifying, or epistatic factors, which potentially modulate the phenotype of SCD do occur and more efforts should be done trying to chart them. The implementation of primary prevention with simple cost effective interventions for SCD are essential (30) and are likely to lead to lower incidence, lower costs for public health and improved survival rate of SCD patients in Oman.

ACKNOWLEDGEMENTS

The authors declare to have conducted this study according to local ethical regulations and to have no conflicts of interest on the presented matters.

REFERENCES

1. Herrick JB. Peculiar elongated and sickle-shape red blood corpuscles in a case of severe anemia. Arch Intern Med 1910; 6: 517-21.
2. Ronald L. Nagel, Shahina Daar, Jose R. Romero, Sandra M. Suzuka, David Gravell, Eric Bouhassira, Robert S. Schwartz, Mary E. Fabry and Rajagopal Krishnamoorthy. HbS-Oman Heterozygote: A New Dominant Sickle Syndrome. Blood. 1998; 92: 4375-4382.
3. Inati A, Taher A, Bou Alawi W, Koussa S, Kaspar H, Shbaklo H, Zalloua PA. b-Globin gene cluster haplotypes and HbF levels are not the only modulators of sickle cell disease in Lebanon. Eur J Haematol 2003; 70: 79–83.
4. Giordano PC, Huisman W, Harteveld CL. Iron depletion: an ameliorating factor for sickle cell disease? ISRN Hematol. 2011;2011:473152.
5. Kamel K. Heterogeneity of sickle cell anaemia in Arabs: review of cases with various amounts of fetal haemoglobin. J Med Genet 1979;16:428–430.
6. Pagnier J, Mears JG, Dunda-Belkhodja O, Schaefer-Rego KE, Beldjord C, Nagel RL, Labie D: Evidence for the multicentric origin of the sickle cell hemoglobin gene in Africa. Proc Natl Acad Sci USA 1984; 81: 1771–1773.
7. Nagel RL, Fleming AF: Genetic epidemiology of the beta s gene. Baillieres Clin Haematol 1992; 5: 331–365.
8. Kulozik AE, Thein SL, Kar BC, Wainscoat JS, Serjeant GR, Weatherall DJ. Raised Hb F levels in sickle cell disease are caused by a determinant linked to the beta globin gene cluster. Prog Clin Biol Res 1987;251:427–439.
9. Padmos MA, Roberts GT, Sackey K, Kulozik A, Bail S, Morris JS, Serjeant BE, Serjeant GR: Two different

forms of homozygous sickle cell disease occur in Saudi Arabia. Br J Haematol 1991; 79: 93–98.

10. Charache S, Terrin ML, Moore RD, et al. Effect of hydroxyurea on the frequency of painful crises in sickle cell anemia. N Engl J Med 1995;332: 1317-22.
11. Walters MC, Patience M, Leisenring W, et al. Bone marrow transplantation for sickle cell disease. N Engl J Med 1996;335:369-76.
12. Van Delft P, Lenters E, Bakker-Verweij M, de Korte M, Baylan U, Harteveld CL, Giordano PC. Evaluating five dedicated automatic devices for haemoglobinopathy diagnostics in multi-ethnic populations. Int J Lab Hematol. 2009 Oct;31(5):484-95.
13. Hassan SM, Hamza N, Jaffer Al-Lawatiya F, Jaffer Mohammed A, Harteveld CL, Rajab A,Giordano PC. Extended molecular spectrum of beta- and alpha-thalassemia in Oman. Hemoglobin. 2010 Jan;34(2):127-34.
14. Jesse Montgomery, Carl T Wittwer, Robert Palais & Luming Zhou. Simultaneous mutation scanning and genotyping by high-resolution DNA melting analysis. Nature Protocoles. 2007; Vol.2 (1): 59-66.
15. Montgomery J, Wittwer CT, Palais R, Zhou L. Simultaneous mutation scanning and genotyping by high-resolution DNA melting analysis. Nat Protoc 2007; 2(1):59-66.
16. Shahina Daar, H. Mohamed Hussain,David Gravell, Ronald L. Nagel and Rajagopal Krishnamoorthy. Genetic Epidemiology of HbS in Oman: Multicentric Origin for the bS Gene. American Journal of Hematology. 2000; 64:39–46.
17. Rajab A, Patton MA. Major factors determining the frequencies of hemoglobinopathies in Oman. Am J Med Genet (Letter). 1997;71: 240–242
18. M. H. Steinberg. Predicting clinical severity in sickle cell anaemia. British Journal of Haematology. 2005; 129: 465–481.
19. Lamk Al Lamki. Deaths from Sickle Cell Disease in Intensive Care Units. Sultan Qaboos Univ Med J. 2012 May; 12(2): 133–136.
20. Steinberg MH, Hsu H, Nagel RL, Milner PF, Adams JG, Benjamin L, Fryd S, Gillette P, Gilman J, Josifovska O, Hellman-Erlingsson S, Safaya S, Huey L, Rieder RF. Gender and haplotype effects upon hematological manifestations of adult sickle cell anaemia: effects of haplotype in sickle cell anaemia. Am J Hematol 1995;48:175–181.
21. Nagel RL, Fabry ME, Pagnier J, Zohoun I, Wajcman H, Baudin V, Labie D. Hematologically and genetically distinct forms of sickle cell anemia in Africa: the Senegal type and the Benin type. New Eng J Med 1985; 312:880–884.
22. Bakanay, S.M., Dainer, E., Clair, B., Adekile, A., Daitch, L., Wells, L., Holley, L., Smith, D. & Kutlar, A. Mortality in sickle cell patients on hydroxyurea therapy. Blood. 2005; 105: 545–547.
23. Nagel, R.L. & Steinberg, M.H. Genetics of the bS gene: origins, epidemiology, and epistasis. In: Disorders of Hemoglobin: Genetics, Pathophysiology, and Clinical Management (ed. by M. H. Steinberg, B. G. Forget, D. R. Higgs & R. L. Nagel). 2001; 711–755. Cambridge University Press, Cambridge.
24. Bailey K, Morris JS, Thomas P, Serjeant GR. Fetal haemoglobin and early manifestations of homozygous sickle cell disease. Arch Dis Child 1992;67:517–520.
25. Bakioglu I, Hattori Y, Kutlar A, Mathew C, Huisman THJ. Five adults with mild sickle cell anaemia share a bS chromosome with the same haplotype. Am J Hematol 1985;20:297–300.
26. Powars DR, Weiss JN, Chan LS, Schroeder WA. Is there a threshold level of fetal hemoglobin that ameliorates morbidity in sickle cell anemia? Blood. 1984; 63:921-926.
27. Acquaye JK, Omer A, Ganeshaguru K, Sejeny SA, Hoffbrand AV. Non-benign sickle cell anaemia in western Saudi Arabia. Br J Haematol 1985;60:99–108.
28. Seltzer WK, Abshire TC, Lane PA, Roloff JS, Githens JH. Molecular genetic studies in black families with sickle cell anemia and unusually high levels of fetal hemoglobin. Hemoglobin 1992;16:363–377.
29. Alexander, N., Higgs, D., Dover, G. & Serjeant, G.R. Are there clinical phenotypes of homozygous sickle cell disease? British Journal of Haematology. 2004: 126, 606–611.
30. Giordano PC. Prospective and retrospective primary prevention of hemoglobinopathies in multiethnic societies. Clin Biochem. 2009;42(18):1757-66.
31. Marion Phylipsen, Supawadee Yamsri, Emmely E. Treffers, Diahann T. S. L. Jansen, Warsha A. Kanhai, Elles M. J. Boon, Piero C. Giordano, Supan Fucharoen, Egbert Bakker and Cornelis L. Harteveld. Non-invasive prenatal diagnosis of beta-thalassemia and sickle-cell disease using pyrophosphorolysis-activated polymerization and melting curve analysis. Prenatal Diagnosis 2012, 32, 578–587.
32. Zago MA, Silva WA, Gualandro S, Yokomizu IK, Araujo AG, Tavela MH, Gerard N, Krishnamoorthy and Elion J. Rearrangements of the beta-globin gene cluster in apparently typical betaS haplotypes. Haematologica 2001; 86:142-145.