CHAPTER 11

SICKLE CELL ANEMIA AND α-THALASSEMIA: A MODULATING FACTOR IN HOMOZYGOUS HBS/S PATIENTS IN OMAN

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Europ J of Med Gen. 2014; 57(11-12):603-606

ABSTRACT

We report the general phenotype severity and the hematological presentation in a cohort of 125 sickle cell anemia (SCA) patients with identical homozygous HbS/S genotype and categorized by identical β^S haplotype, both with and without alpha thalassemia. No clear general phenotype correlation was found when patients were compared regardless of the haplotype but overall, patients with homozygous alpha thalassemia (α−/α−) had the highest Hb, HCT, RBC and the lowest MCV, MCH and MCHC levels. When patients with identical haplotype were compared, the mildest hematological and clinical conditions were observed in patients of the Asian/Asian haplotype, also known as Arab-Indian haplotype, and carriers of α-thalassemia, suggesting an additional ameliorating effect of alpha thalassemia. In conclusion, our results show that alpha thalassemia improves the hematological conditions but amelioration of the general disease severity is only noticed when compared in cohorts of the same haplotype.

INTRODUCTION

Sickle cell anemia (SCA) is in general a severe condition caused by different genotype combinations of which HbS homozygosis is the most common. The pathophysiology of SCA is complex and involves HbS polymerization in hypoxic conditions in the post-capillary veins, erythrocyte sickling, chronic and acute vaso-occlusive events, hemolysis and progressive organ and tissue damage at variable levels (1). Although it is known that both environmental and genetic factors may contribute to this variability (2), patients with the same HbS/S genotype often display very different phenotypes in which the clinical manifestations may range from very severe to milder or can even in some cases be almost asymptomatic and be diagnosed accidentally (3,4). The clinical and hematological severity of SCA can be influenced by a number of factors among which the main one is the level of fetal hemoglobin (HbF) in postnatal life which is related to the beta globin gene haplotype (5). The role of co-inherited alpha thalassemia influencing or not the phenotype of SCA has been long debated (6).

The alpha-globin chains that are needed to form sufficient Hb tetramers are coded by two alpha genes located on the short arm of chromosome 16. The two α-globin genes, alpha 2 and alpha 1, are separated by less than 4 kb and code for identical alpha globin chains.

Alpha thalassemia occurs at a high prevalence in Oman (7) with -α3.7 kb deletion being the commonest (8).

The influence of alpha-thalassemia on SCA has been reported to ameliorate the hematological and clinical manifestation of the disease in several populations (9). Alpha-thalassemia lowers the mean cell volume (MCV) and the mean cell hemoglobin (MCH), and both these changes might be expected to be beneficial to patients with sickle-cell disease improving rheology and reducing the concentration of the Hb molecules in the red cells (2). However, to study the effect of alpha thalassemia on the severity of the disease one needs to compare cohorts of patients with identical genotype (HbS/S mutation) and haplotype (beta globin cluster).

Both alpha thalassemia and SCA are frequent in Oman (7) and the HbS mutation is present on severe and mild haplotypes. Therefore, in order to establish if alpha thalassemia has any effect on the severity of the disease, we have studied the hematological and general clinical phenotype of 125 patients, all HbS/S homozygous, with or without alpha thalassemia and categorized in specific and different haplotypes. We premise therefore that our study is not meant to go deeply into clinical details. Our goal is to build a bridge between the clinician and the geneticists, comparing the general clinical phenotype with the genetic background of the patients.

MATERIALS AND METHODS

Blood samples were collected in EDTA at the Ministry of Health Hospitals in Oman. Hematological and clinical data were obtained from routine hematology and from patient's medical history documented by the treating physician. We subdivided the phenotypes as mild, intermediate and severe by the occurrence of symptoms such as: acute chest syndrome, stroke and the presence of avascular necrosis (absence = mild, presence = severe); number of painful crisis per year (≤3 = mild, ≥6 = severe) and blood transfusion (2/year≤ = mild, 4/year≥ = severe).

The cohort consisted of individuals that had been previously diagnosed with SCA. The diagnosis was confirmed on HPLC (Variant II, Bio-Rad Laboratories, Hercules, CA, USA) (10) and molecular characterization of the genotypes and haplotypes was performed at Leiden University Hemoglobinopathies center. A group of 125 individuals confirmed homozygous HbS/S were enrolled in this study. The median age was 24 years. DNA extraction was done using the commercial Qiagen kit as per the manufacturer instruction (8). The beta genotype was confirmed by direct Sanger DNA sequencing. The haplotype of the β-globin gene cluster was determined by melting curve analysis as previously reported (11) and genotyping errors were ruled out by random sample sequencing. We found a Central African Republic (CAR) derivative haplotype in some patients and named it the Oman haplotype. This haplotype differs from CAR by a single variation at HcII RFLP G>A (SNP F5, position rs968857 5260458) (11). Five patients were not included in the haplotype grouping analysis because each had a single unique haplotype with no comparable cases. Alpha-globin genotype was established by GAP-PCR for the most common 7 alpha thalassemia deletion defects (12) for all the samples while the alpha globin genes were sequenced for selected samples. To asses if presence or absence of alpha thalassemia has an effect on disease expression, clinical and hematological comparison of the patient's history was made between genetically equivalent cohorts with and without alpha thalassemia.

RESULTS

α-thalassemia's frequency in the different cohorts

The gene frequency of α-thalassemia among HbS/S patient was confirmed to be very high. Homozygosis or compound heterozygosis (-α/-α), was found in 55 patients (44%). Specifically 54 had the ($-\alpha^{3.7}/-\alpha^{3.7}$) and 1 had the ($-\alpha^{3.7}/-\alpha^{4.2}$) combination while 42 patients (33.6%) had the heterozygous genotypes ($-\alpha^{3.7}/\alpha\alpha$) and 28 individuals (22.4%) had a normal alpha globin genotypes (αα/αα). No alpha° deletions or point mutations were found in the studied samples.

When HbS/S patients classified as mild, intermediate and severe, based upon their disease history, were compared with the presence or absence of α-thalassemia regardless the haplotype, no clear correlation was found.

Effect of α-thalassemia in HbS/S patients of specific haplotypes

HbS/S patients with Asian/Asian haplotype (also known as Arab-Indian) had a mild presentation in 82% and 87% of the cases with (-α/-α) and (-α/αα) thalassemia respectively. This in contrast with 66% of the cases without alpha thalassemia. Similarly, an intermediate state, was twice more frequent in absence of alpha thalassemia. None of the Asian/Asian presented with a severe condition (Table 11.1a).

In the smaller cohorts of HbS/S patients with Asian/Oman haplotype, the milder condition was present in 75% of the patients with (-α/-α) while none had the mild condition in absence of alpha thalassemia and none of them were severe (Table 11.1b).

In the few HbS/S patients with Asian/CAR haplotype and (-α/-α) all three conditions (mild, intermediate and severe) were observed but the severe phenotype was 3 time higher in absence of alpha thalassemia (Table 11.1c).

Table 11.1. Association between alpha-thalassemia, different β-cluster haplotypes and clinical severity.

(a)Asian/Asian haplotype

Genotype	Mild	Intermediate	Severe
-α/- α (n=20)	82.4%	17.6%	-
- α/αα (n=9)	87.5%	12.5%	-
αα/ αα (n=18)	66.7%	33.3%	-

(b)Asian/Oman haplotype

Genotype	Mild	Intermediate	Severe
-α/- α (n=5)	75%	25%	-
- α/αα (n=7)	57.1%	42.9%	-
αα/ αα (n=2)	-	100%	-

(c)Asian/CAR haplotype

Genotype	Mild	Intermediate	Severe
-α/- α (n=3)	33.4%	33.3%	33.3%
- α/αα (n=3)	-	66.7%	33.3%
αα/ αα (n=2)	-	-	100%

(d)Benin/Benin haplotype

Genotype	Mild	Intermediate	Severe
-α/- α (n=16)	-	25%	75%
- α/αα (n=8)	-	57.1%	42.9%
αα/ αα (n=1)	-	-	100%

(e)CAR/Oman haplotype

Genotype	Mild	Intermediate	Severe
-α/- α (n=5)	-	40%	60%
- α/αα (n=6)	-	16.7%	83.3%
αα/ αα (n=1)	-	-	100%

(f)CAR/CAR haplotype

Genotype	Mild	Intermediate	Severe
-α/- α (n=4)	-	-	100%
- α/αα (n=5)	-	20%	80%
αα/ αα (n=4)	-	-	100%

In spite of the limited number of cases found on other haplotypes, the same pattern seems to appear in the Benin/Benin and in the CAR/Oman cohorts (Table 11.1d and 11.1e) while no association seems to be present in the severe CAR/CAR cohort (Table 11.1f).

Effect of α-thalassemia in grouped HbS/S patients of mild and severe haplotypes

HbS/S patients with Asian/Asian and Asian/Oman were grouped under mild haplotype while those with Benin/Benin, CAR/Oman and CAR/CAR under severe haplotype. Asian/CAR was not included in the 2 groups as it had an intermediate phenotype. Patients grouped as mild haplotype had a mild presentation in cases with (-α/-α) and (-α/αα) thalassemia than cases without alpha thalassemia (Table 11.2a). Among the severe haplotype, all patients without alpha thalassemia were presented with a severer phenotype (Table 11.2b).

Table 11.2. Association between alpha-thalassemia, mild and severe β-cluster haplotypes and clinical severity.

(a) Mild haplotype

Genotype	Mild	Intermediate	Severe
-α/- α (n=25)	77.3%	22.7%	-
- α/αα (n=16)	73.3%	26.7%	-
αα/ αα (n=20)	60%	40%	-

(b) Severe haplotype

Genotype	Mild	Intermediate	Severe
-α/- α (n=25)	-	24%	76%
- α/αα (n=19)	-	31.6%	68.4%
αα/ αα (n=6)	-	-	100%

Effect of α –thalassemia on the hematological parameters of HbS/S patients

The presence of alpha-thalassaemia homozygosis (-α/-α) resulted in significantly higher mean hemoglobin (Hb) levels, hematocrit (HCT), red blood cells counts (RBC) but lower levels of fetal hemoglobin (HbF), mean cell volume (MCV), mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) than the group with a normal alpha genotype (αα/αα). Patients with heterozygous alpha complement (–α/αα) showed intermediate mean hematological values. The overall distributions of hematologic parameters in Hb S/S patients with three different alpha-globin genotypes are summarized in Table 11.3.

Table 11.3. The effects of the various α-thalassemia genotypes on the mean hematological parameters in HbS/S patients regardless of the haplotype.

	Hb F(%)	Hb (g/dl)	HCT (%)	RBC (x10^{12}/L)	MCV (fl)	MCH (pg)	MCHC (g/dl)
-α/-α (n=55)	10.4	9.7	29.2	4.2	69.3	22.9	33.2
-α/αα (n=42)	10.8	9.1	26.7	3.3	79.9	27.3	34.2
αα/αα (n=28)	15.9	9.0	25.8	3.1	84.8	29.8	35.1

Influence of α-thalassemia on hematological parameters in HbS/S patients of different haplotypes

Alpha-thalassemia increased the mean Hb, HCT, RBC and lowerd HbF, MCV, MCH and MCHC in patients with Asian/Asian, CAR/Oman and CAR/CAR haplotypes (Table 11.4 a, e and f). In patients with Asian/Oman and Asian/CAR haplotypes the HbF level was however found increased (Table 11.4 b and c). In HbS/S patients of the severe Benin/Benin haplotype only a decreased MCV and MCH were observed. Data are summarized in Table 11.4.

Table 11.4. Effects of various α-thalassemia genotypes on the average hematological parameters in HbS/S patients based on their haplotype:

(a)Asian/Asian haplotype

	Hb F(%)	Hb (g/dl)	HCT (%)	RBC (x10^{12}/L)	MCV (fl)	MCH (pg)	MCHC (g/dl)
-α/- α (n=20)	13.5	9.6	29.1	4.1	69.9	23.1	33.1
- α/αα (n=9)	18.4	9.6	27.8	3.7	74.9	25.7	34.3
αα/ αα (n=18)	20.4	9.2	26.2	3.1	83.9	29.6	35.2

(b)Asian/Oman haplotype

	Hb F(%)	Hb (g/dl)	HCT (%)	RBC (x10^{12}/L)	MCV (fl)	MCH (pg)	MCHC (g/dl)
-α/- α (n=5)	9.7	11.2	33.5	5.0	66.6	22.3	33.4
- α/αα (n=7)	17.3	10.6	31.8	3.9	79.8	26.7	33.5
αα/ αα (n=2)	9.2	8.4	23.5	2.8	82.1	29.7	35.6

(c)Asian/CAR haplotype

	Hb F(%)	Hb (g/dl)	HCT (%)	RBC (x10^{12}/L)	MCV (fl)	MCH (pg)	MCHC (g/dl)
-α/- α (n=3)	12.7	10.1	29.9	4.1	74.5	25.2	33.7
- α/αα (n=3)	14.1	8.1	23.9	3.1	77.7	26.5	34.0
αα/ αα (n=2)	10.4	9.5	26.6	2.9	90.8	32.4	35.7

Table 11.4. Effects of various α-thalassemia genotypes on the average hematological parameters in HbS/S patients based on their haplotype (*Continued*):

(d)Benin/Benin haplotype

	Hb F(%)	Hb (g/dl)	HCT (%)	RBC (x10^{12}/L)	MCV (fl)	MCH (pg)	MCHC (g/dl)
-α/- α (n=16)	5.9	9.3	28.5	4.0	69.2	22.7	32.9
- α/αα (n=8)	3.6	8.7	26.1	3.4	76.2	25.5	33.4
αα/ αα (n=1)	4.2	11.0	34.6	4.3	80.1	25.6	32.0

(e)CAR/Oman haplotype

	Hb F(%)	Hb (g/dl)	HCT (%)	RBC (x10^{12}/L)	MCV (fl)	MCH (pg)	MCHC (g/dl)
-α/- α (n=5)	4.7	9.3	28.5	3.9	72	23.3	32.6
- α/αα (n=6)	5.3	8.3	23.7	2.8	84.9	29.3	34.6
αα/ αα (n=1)	5.0	9.1	25.8	2.9	87.9	31.0	35.3

(f)CAR/CAR haplotype

	Hb F(%)	Hb (g/dl)	HCT (%)	RBC (x10^{12}/L)	MCV (fl)	MCH (pg)	MCHC (g/dl)
-α/- α (n=4)	4.6	9.2	27.4	3.9	68.7	23.1	33.6
- α/αα (n=5)	5.8	7.5	21.6	2.5	86.2	30.1	34.9
αα/ αα (n=4)	7.5	7.6	22.1	2.4	89.3	30.8	34.5

DISCUSSION

When performing correlation studies between genotype and phenotype it is important to compare cohorts that are not only phenotypically similar but genotypically identical in order to reduce genetic variables to a minimum. Therefore we have selected groups with identical genotypes and haplotypes with and without alpha thalassemia. Although external and accidental factors cannot be avoided, we do believe that our cohorts are as comparable as possible. As mentioned in the introduction, our study is not meant to be a detailed clinical report but rather a correlation study based upon the occurrence of general symptoms that may indicate mild, intermediate or severe conditions.

The frequency of alpha thalassemia in the present cohort (72%) was higher than the (58.3%) measured by our self in a previous study (8) and by AlKindi et al (48.5%) (7). This is probably due to a bias deriving by the selection of homozygous HbS/S patients with a much higher chance of being the progeny of consanguineous parents.

Previous and present studies

In a similar study reported by Mukherjee et al., all SCA patients from Western India with homozygous α-thalassemia had a mild phenotype (13). The clinical presentation of our total cohort with homozygous alpha-thalassemia ranged from mild (38.2%), to intermediate (21.8%) and to severe (40%) cases. Mukherjee et al. studied patients of the Asian/Asian haplotype

with high HbF levels (13, 14) and our data on the 47 patients with Asian/Asian haplotype shows that among cases with (-α/-α), 82.4 % were mild and 17.6% intermediate while no severe cases were present. Considering the variability of the definition "intermediate" our results are quite compatible with the observations of the Indian study and with the conclusions of other authors reporting milder conditions among Saudis and Kuwaitis carrying the Asian haplotype with α -thalassaemia, when compared with Asian haplotypes without α-thalassaemia (15). The same correlation was also observed in patients with Asian/Oman haplotype and (-α/-α) in which 75% had a mild disease and 25% were intermediate. On the other hand, our results also show that alpha thalassemia, although ameliorating the hematological parameters, is of little effect in reducing the symptoms of the HbS/S homozygous with the severe CAR and Benin haplotypes.

Hematological data and HbF

Alpha thalassemia is believed to improve the survival of the erythrocytes in SCA resulting in a milder form of anaemia due to decreased hemolysis (16). How relative can be the effect of alpha thalassemia in SCA is shown by many contrasting reports (17). In our study, the presence of alpha-thalassemia in Hb S/S homozygotes resulted in significantly higher mean Hb and HCT levels as well as higher RBC counts and these three parameters indicate a better RBC survival as a consequence of lower hemolysis. In addition, patients with homozygous alpha-thalassemia had on average lower levels of fetal Hb (HbF) and a lower MCHC than patients with a normal alpha globin genotype (αα/αα).

In our study the reduction of HbF expression was particularly relevant in patients with the mild "high HbF" (Asian) haplotypes and (-α/-α) alpha thalassemia. Conversely, in patients with severe "low HbF" haplotypes (CAR and Benin) the HbF remained either unchanged or increased in the presence of alpha thalassemia while little changes were observed in these patients also at the RBC level. Other studies have shown that coexistence of α-thalassemia enhances the levels of HbF associated with a specific haplotype such as the Senegal (18) and Benin haplotypes (19).

Higgs et al. (20) reported a decrease in the level of HbF in SCA patients with homozygous alpha-thalassemia while Embury et al. (21) reported an increase in HbF levels (9).

Higgs et al. (20) also observed that (-α/-α) individuals had the lowest cell volume and hemoglobin content per cell (MCHC) which reduce the polymerization risk of the Hb molecules in the smaller and less dense cells. In addition, low MCV in (-α/-α) generates a relatively larger cellular surface compared to (αα/αα) which might give the cells the property of increased membrane redundancy, providing a larger reserve of internal volume per given amount of polymer and thus giving protection against the deleterious consequences of membrane stretching during deoxygenation (21). We do believe that smaller cells (low MCV) might just be faster, passing the risk area of the post-capillary veins as this seems to be the same rheological advantage observed in mild SCA phenotypes with microcytic hypochromic parameters due to iron deficiency (12).

In conclusion the lower MCV, MCH and MCHC associated with alpha-thalassemia should diminish the amount of intravascular sickling while the decreased intra-erythrocytic hemoglobin S concentration associated with α-thalassemia should diminish polymerization (sickling) and herewith the degree of chronic hemolytic anemia typical of SCA (17). However, other factors (both genetic and environmental) are involved making the interpretation of phenotype/genotype association studies more complex.

CONCLUSION

Although some of the haplotype cohorts are small, it seems evident from the present study that alpha-thalassaemia can modulate the hematological picture of HbS/S but not clearly the overall severity manifestation in patients of all haplotypes. However, when the cohort was subdivided into larger groups of similar haplotypes, differences in the mild and severe forms became more evident. In addition, variability in clinical outcomes of sickle cell disease is not modulated by genetic factors alone, but also by environmental factors and life style.

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