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CHAPTER 10

ASSOCIATION OF XMNI (-158 γ^G)
POLYMORPHISM AND RESPONSE
TO HYDROXYUREA IN OMANI S/S
AND S/β PATIENTS

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ABSTRACT

Objective

To describe the effect of hydroxyurea (HU) treatment in Omani sickle cell disease (SCD) patients with different beta-globin gene cluster haplotypes.

Materials and Methods

A total of 52 cases treated with HU were enrolled in this study. Response to the drug was compared between patients with and without the XmnI polymorphism in the different beta-globin gene cluster haplotypes. We have classified our cohort into three categories: good responders to HU for those patients who had no crises and no hospitalization after 6 months of treatment; partial responders for those who had a reduction in the number of crises after the same period and non responders for those that remained clinically unchanged even after doubling the HU prescription.

Results

Most patients homozygous or heterozygous for the Xmn I polymorphism (T/T or T/C) had higher levels of HbF prior treatment than those having the CC genotype and were classified under good or partial responders.

Conclusions

Being the Xmn-I polymorphism associated with the haplotypes frequent in Oman and acting as enhancer of the already elevated HbF expression, HU treatment can be prospectively applied to predict responsiveness and treatment can be given to those with low HbF expression for beneficial lowering of cellular adhesion. HU treatment can ameliorate the clinical phenotype of the large majority of Omani patients with SCD.

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ASSOCIATION OF XMNI (-158 γ⁹) POLYMORPHISM

INTRODUCTION

The mutation responsible for sickle cell disease (SCD) is the GAG > GTG transversion at codon 6 of the beta globin gene, resulting into a Glu>Val amino acid substitution and in the change of the wild type HbA tetramer into the commonest abnormal Hb variant (HbS). The condition is recessive and carriers of HbS are in general asymptomatic while the mutation in homozygous or compound heterozygous form in combination with β-thalassemia is in most of the cases a severe condition. Although HbS is the causal allele, the combination of HbS with other common alleles (HbC, HbE, HbD) and a number of less common ones, may also cause the disease with large phenotype variability (1).

Milder forms may be caused by the combination of HbS with a less severe β⁺-thalassemia allele with residual HbA expression or with δβ-thalassemia deletion because of the characteristic elevated HbF expression. The ameliorating role of HbF in SCD and β-thalassemia and the association of high HbF with specific genotypes / haplotypes have been known for a long time. In spite of strenuous efforts, no effective cure associated with a permanent increase of HbF expression in post-natal life has been found so far while different drugs have been tested that can temporarily increase the HbF level with acceptable collateral effects. Among these drugs the most successful thus far is hydroxyurea (HU) a medication that may reduce the severe symptoms in different ways in SCD patients (2). A better practical knowledge on these differences may allow an early prognosis for severe patients that are likely to respond to HU and for others that are not responding or respond in a different way, allowing early planning for an alternative treatment for non-responders such as bone marrow transplantation which, if successful, could be an alternative curative solution (3).

Response to HU has been shown to be largely associated with the presence of the C>T polymorphism at -158 Xmn-I site (HBG2:c.-53-158C>T) upstream of the Gγ globin gene and it is thus far the most studied nucleotide change to have a significant association to drug response. This particular polymorphism acts as an enhancer of HbF expression during erythropoietic stress, resulting in a beneficial effect in SCD patients (4). The frequency of Xmn-I polymorphism in SCD patients has not yet been investigated in the Omani population. Therefore we have studied the association of the XmnI polymorphism and the response to hydroxyurea treatment in HbS/S and S/β-thalassemia patients in Oman and we have further investigated if the HbS haplotype is accountable for a more differentiated response to HU in patients with identical Xmn-I genotype.

MATERIALS AND METHODS

A total of 52 SCD patients attending the Ministry of Health Hospitals in Oman (between January – June 2012) and started the treatment with HU were randomly enrolled. These patients were then followed for the subsequent 6 months afterwards. Gender distribution was; 52% males and 48% females. The age range of the subjects was 23- 32 years. At the laboratory level, high performance liquid chromatography (HPLC) was performed on all samples, prior treatment, either on D-10 (short and extended programs) device and/or the Variant II (Bio-Rad Laboratories, Hercules, CA, USA). DNA was extracted using the QIAamp DNA Blood Mini Kit (Qiagen Inc., Valencia,

CA, USA). The XmnI (−158 (C>T) polymorphism of the Gγ gene promoter) (rs 7842144, HBG2:c.-53-158C>T) and the haplotype of the HbS/S beta globin cluster were defined by melting curve analysis (LightScanner HR96, Idaho Technology, Inc) as previously described (5). The β globin gene sequencing was performed on an ABI PRISM® 3700 DNA Analyzer (PE Biosystems, Foster City, CA, USA) and the α-cluster genotype was obtained by GAP-PCR as previously described (6). The initial hydroxyurea dosage was 500 mg (capsule). If no improvement was observed after 3 months the dosage was increased to 1000 mg. Classification of responders was as follow: Good responders: Patients became clinically asymptomatic 6 months after the therapy (no crises, no hospitalization, no definitive pain). Partial responders: Significant decrease is noted in number of vaso-occlusive crises and hospitalizations (<3/year) and experienced much milder pain. Non responders: No clinical improvement despite dosage increase after 6 months.

RESULTS

DNA analysis revealed 40 cases homozygous for HbS/S and 12 S/β-thalassemia patients. Of these 52 patients, 22 were either homozygous or heterozygous for the Xmn I (C>T) polymorphism at position -158 site at the G-γ promoter. Of the patients homo- or heterozygous for the -158 C>T, 18 (82%) showed good response to HU, 3 (13.6%) partial response and 1 (4.5%) showed no response. In those without Xmn I polymorphism (n=30), only 1 (3.3%) showed good response, 24 (80%) showed partial response and 5 (16.7%) showed no response. On average, good responders became asymptomatic after 6 months of being on HU, partial responders had significant decrease in complications within the same time span while non-responders had no significant reduction after 6 month even after doubling the HU dosage. Among the 40 HbS/S patients, 15 that had at least one copy of the T allele, were all good responders while among those 21 that became better (partial responders) only two had at least one copy of the T allele. Of the remaining 4 patients that did not respond, even after double prescription, none had the T allele.

Prior to treatment, HbS/S patients with the genotype T/T or T/C had on average an elevated expression of HbF% (15.2%) and (13.5%) respectively. Patients having the genotype C/C had a lower HbF level (5.6%).

None of the S/β-thal patients, had the TT genotype, however, HbF levels prior treatment were found to be on average high in S/β individuals bearing the TC genotype (11.3%) and lower in those with the CC genotype (7.3%). Among the S/β-thal group, we found 4 patients that were good responders to HU, 6 became better in terms of severity while 2 did not respond to the drug. Data are summarised in Table 10.1. One of the non responders was found to have the Xmn I T/C polymorphism and the β IVS-I-1 G>A mutation while the other had the C/C polymorphism and the β Cd36/37 (-T) mutation. Data are summarized in Table 10.2. Over all, the clinical symptoms improved in most patients and the best response was associated with the presence of the Xmn I polymorphism.

Haplotype analysis of the HbS/S is summarised in Table 10.3. The 4 non responders all had the CAR/Oman haplotype. The Oman haplotype differs from CAR by a mutation at SNP position 49994 (4). Moreover, the presence of the T allele at the XmnI polymorphism was linked to the Asian haplotype. Our findings show that the presence of Xmn I polymorphism in Omani SCD population is a predictor of response to HU.

Table 10.1. Association between improvement of disease after HU treatment and the presence of the polymorphic site (C>T) at position -158 Xmn I at the G-γ promoter region in the S/S (i) and S/β (ii) cohorts.

(i)

S/S patients	T/T (%) (n=11)	T/C (%) (n=6)	C/C (%) (n=23)
Good responders	91% (n=10)	83.3% (n=5)	-
Partial responders	9% (n=1)	16.7% (n=1)	82.6% (n=19)
Non responders	-	-	17.4% (n=4)

(ii)

S/β patients	T/C (%) (n=5)	C/C (%) (n=7)
Good responders	60% (n=3)	14.3% (n=1)
Partial responders	20% (n=1)	71.4% (n=5)
Non responders	20% (n=1)	14.3% (n=1)

Table 10.2. Genotypes of the HbS/β compound heterozygote patients treated with HU.

β genotype	XmnI genotype	response to HU
Cd6 GAG>GTG/IVS-I-5 G>C (n=7)	T/C (n=2)	Excellent (n=2)
HBB:c.20A>T/ HBB:c.92+5G>C		Partial (n=0)
		None (n=0)
	C/C (n=5)	Excellent (n=1)
		Partial (n=4)
		None (n=0)
Cd6 GAG>GTG/Cd121 GAA>CAA (n=2)	T/C (n=1)	Excellent (n=1)
HBB:c.20A>T/ HBB:c.364G>C		Partial (n=0)
		None (n=0)
	C/C (n=1)	Excellent (n=0)
		Partial (n=1)
		None (n=0)
Cd6 GAG>GTG/Cd44 (-C) (n=1)	T/C (n=1)	Excellent (n=0)
HBB:c.20A>T/ HBB:c.135delC		Partial (n=1)
		None (n=0)
Cd6 GAG>GTG/IVS-I-1 G>A (n=1)	T/C (n=1)	Excellent (n=0)
HBB:c.20A>T/ HBB:c.92+1G>A		Partial (n=0)
		none (n=1)
Cd6 GAG>GTG/Cd37(-T) (n=1)	C/C (n=1)	Excellent (n=0)
HBB:c.20A>T/ HBB:c.112delT		Partial (n=1)
		none (n=1)

Table 10.3. Summary of the HbS/S haplotypes in patients treated with HU.

Haplotype	XmnI genotype	response to HU
Asian/Asian (n=11)	T/T	Good (n=10)
		Partial (n=1)
		None (n=0)
Asian/CAR (n=1)	T/C	Good (n=1)
		Partial (n=0)
		None (n=0)
Asian/Oman (n=4)	T/C	Good (n=3)
		Partial (n=1)
		None (n=0)
Senegal/Oman (n=1)	T/C	Good (n=1)
		Partial (n=0)
		None (n=0)
Benin/Benin (n=9)	C/C	Good (n=0)
		Partial (n=9)
		None (n=0)
CAR/CAR (n=3)	C/C	Good (n=0)
		Partial (n=3)
		None (n=0)
CAR/Oman (n=11)	C/C	Good (n=0)
		Partial (n=7)
		None (n=4)

DISCUSSION

Bakanay et al. reported the highest incidence of organ damage and the poorest response to HU in SCD patients with the XmnI C/C genotype (7). Likewise, our patients bearing the C/C allele at the XmnI site, had a poorer response to the drug than those carrying the T allele.

In patients with the genotype TT, high HbF levels and the best response to HU were measured as previously reported by other authors (8) with significant reduction in both anaemia and the frequency of vaso-occlusive events (9). Studies on HU treatment for β-thalassemia have produced contradictory results. While Karimi et al. found no relationship between XmnI polymorphism and HU clinical response in their patients (10), Yavarian et al. (4) found that the C>T polymorphism at position -158 of the Gγ promoter was the most significant parameter correlating with HU response in β-thalassemia patients. In our study as many as 91% of the HbS/S patients with the T/T genotype fully responded to HU therapy while none of the patients with the C/C polymorphism were classified under 'good responders', confirming that this polymorphism is highly correlated with a positive response to HU treatment in the Omani SCD patients as well.

In Oman, HU is only used for SCD but not for β-thalassemia major and thus far a restricted inclusion criterion have been used to decide if SCD patients were eligible to be given HU or not. These include some severe symptoms noted by the clinician, such as frequent hospitalization (>4-5/year) with recurrent episodes of acute chest syndrome, vaso-occlusive events, severe pain crisis and severe, un-subsiding body and limp pain that last for days. The daily dose given is the recommended 500mg and it is only increased to reach a maximum of 1000mg when no improvement is seen with the 500mg dose. Although Charache et al, proved the effectiveness and safety of hydroxyurea usage and the improved outcomes (11), HU is not widely accepted by Omani patients and their families, due to the negative perception toward this treatment and the fears of birth defects, infertility, malignancy and concerns on long-term risks. Non-compliance may be found in patients because of anxiety and minor but disturbing side effects such as nausea or when a pregnancy is perceived. More studies have proven that HU (20 mg/kg/d) is a safe therapy even for very young children with SCD (starting at 9 months of age) and that the cure is in general associated with significantly lower rates of recurrent episodes of pain, dactylitis, acute chest syndrome and hospitalization (12). However, response prediction to HU treatment is not always straightforward. Also in our study some patients having the T/C XmnI polymorphism, did not improve with the standard 500mg dose which had to be doubled to observe some improvement. The few patients that did not show improvement after treatment with HU even after increasing the dose were all carries of the compound heterozygous haplotype (CAR/Oman). Our findings suggest that the C/T polymorphism at the XmnI site, although in most cases associated with good response, is neither a guarantee nor the only determinant that can ameliorate disease severity in SCD patients treated with HU and that other factors either haplotype and/or sub-haplotype related or associated with other regulatory elements might be involved. Therefore, increasing the dosage of HU to the maximal tolerated dose might be necessary for having some clinical response in SCD patients with African haplotypes (13) whereas SCD children with Asian haplotype treated with a dose as low as 10 mg/kg/day have shown some good clinical response (14). Moreover, it has been proved that HU a nitric oxide (NO) provider has an anti-adherence effect that may prevent cells to get stuck to the capillary walls and herewith improving the rheology of the blood during the critical passage of the deoxygenated HbS cells (15).

Alpha thalassemia has been proposed as a factor possibly associated with good response to HU therapy in β-thalassemia intermedia in addition to the presence of Xmn I polymorphism (16). While another study reported that co-inheritance of α-thalassemia in SCD patients may reduce the clinical response to HU therapy (17). Although we may imagine that α-thalassemia may partially restore the balance in β-thalassemia, we believe that this mechanism has little to do with the response mechanisms of HU in SCD. As a matter of fact when we correlated the presence or absence of α-thalassemia (very common in our cohort) to the HU response (HbS/β-thal included) we find no association between coexisting alpha deletions and good response to HU (data not shown).

In conclusion, being the Xmn-I polymorphism associated with haplotypes frequent in Oman and acting as enhancer of the already elevated HbF expression, HU treatment can be prospectively applied to predictably responsive patients and be tested in those with low HbF

expression for beneficial lowering of cellular adhesion. HU treatment can ameliorate the clinical phenotype of the large majority of Omani patients with SCD.

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