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ORIGINAL ARTICLE

The Effect of Hydroxyurea Therapy in Bahraini Sickle Cell Disease Patients

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Abstract Hydroxyurea (HU) is used as a disease-modifying agent in sickle cell disease (SCD). Its beneficial effects have been ascribed to inhibition of the sickling process through increase of fetal hemoglobin (HbF) levels and influence on multiple factors affecting adhesion of erythrocytes to vascular endothelium. The present study investigates the effect of HU in SCD patients who were grouped on the basis of association with α- and β-thalassemia using routine laboratory methods. A retrospective cross-sectional chart-review was done of 51 adult Bahraini SCD patients attending Salmaniya Medical Complex, Bahrain. Four sub-groups of cases were identified: (i) homozygous sickle cell anemia, 24 cases; (ii) SCD with microcytosis, 16 cases; (iii) sickle α-thalassemia, seven cases; and (iv) sickle β thalassemia, four cases. Documented laboratory and clinical data included hemoglobin level (Hb), hematocrit (Hct), red cell indices, hemoglobin fractions, hospital admissions (frequency), number of inpatient-days, pain episodes (frequency) and red cell transfusion requirement (number of units). Pre- and post-treatment data were compared. Hydroxyurea treatment led to highly significant reduction of HbS % and pain crisis episodes in all patient groups. Other changes such as increases of total hemoglobin, Hct and HbF and reduction of hospital admissions, inpatient days and red cell units transfused also occurred but with less consistent levels of significance within patient sub-groups. Treatment with HU is beneficial for all subgroups of Bahraini SCD patients, without or with α- and β-thalassemia interactions.

Keywords Sickle cell disease · Thalassemia · Hydroxyurea · Fetal hemoglobin

Introduction

Sickle cell disease (SCD) is a genetic disorder of hemoglobin synthesis in which a single amino-acid substitution in the β-globin chain of hemoglobin ($\beta^{6Glu\rightarrow Val}$) leads to formation of hemoglobin S (HbS). Under conditions of hypoxia HbS polymerizes into long rigid arrays called tactoids which results in sickling. Numerous factors have been postulated to influence the rate of sickling: the most commonly implicated ones being oxygen tension, pH, temperature, erythrocyte age and intra-erythrocytic concentrations of HbS and other hemoglobin fractions, principally HbF and HbA [1, 2]. Sickling is directly correlated with the concentration of HbS and inhibited by HbF and HbA. The inhibition of sickling by HbF is greater than that of HbA [1].

Among available treatment options that ameliorate sickling crises, the use of therapeutic agents that increase the levels of HbF, such as hydroxyurea (HU), butyrate and decitabine, have been shown to be beneficial [3]. The initial studies documenting the benefits of HU were published between 1984 and 1995 [4, 5]. The drug was approved for clinical use by the U.S. Food and Drug Administration (*FDA*) in 1998.

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Hydroxyurea is a ribonucleotide reductase inhibitor and arrests cells in S-phase as a result of impairment of DNA replication. The rationale for the use of HU in the treatment of SCD was based on its ability to increase Hb F synthesis.The drug increases intra-erythocytic HbF by stimulating HbF production in primitive erythroid precursors and by killing rapidly dividing late erythroid cells with recruitment of more primitive erythroid precursors that produce high levels of HbF [1, 6].

In addition to increasing the level of HbF, HU also reduces expression of various adhesion molecules on sickle erythrocytes, including very late activation antigen-4 (VLA-4) and CD36 [7, 8]. It reduces leukocyte, platelet and reticulocyte counts and hence decreases adhesive interactions between these cells and the endothelium thereby reducing vaso-occlusive events [6, 9–12]. Neutrophils release powerful pro-inflammatory mediators and cytokines which are important in endothelial damage and could trigger sickling [12]. Lower neutrophil counts following HU administration may limit the extent of tissue destruction following infarction and reduce the severity of pain [9].

Variation of HbF levels in SCD patients may be attributed to genetic interactions such as association with distinct β-globin gene haplotypes. Haplotypes may be markers for linked DNA that modulate gamma-globin gene expression. A number of other genetic modifiers are known to affect the phenotypic expression of this classic "monogenic" disease [13]. There is a high prevalence of hereditary disorders of hemoglobin synthesis in the Arabian Gulf region [14]. In particular, SCD, α-thalassemia and β-thalassemia and their interactions are relatively common and contribute to the phenotypic variability of SCD in this region. Although sickle cell anemia co-inherited with α-thalassemia (SS/α-thal) is traditionally linked to a milder clinical phenotype, numerous studies have shown that this is not necessarily so with respect to specific types of complications. For example, it could be associated with a higher frequency of severe pain episodes [15].

Despite the relatively common occurrence of SCD in the Arabian Gulf region, there are few published reports from this geographic region describing the results of HU therapy in SCD and rarely have these compared the results between subgroups of patients with/without associated thalassemia [16, 17]. Theoretically, sickle cell-thalassemia interactions could be important considerations affecting therapy response because pharmacological agents used to stimulate synthesis of gamma-globin may affect the activity of the other globin genes variably. For example, butyrate was shown to reduce synthesis of α-globin in SCD [18].

Therefore the aim of the present study was to investigate whether there is a differential response to HU in subgroups of Bahraini SCD patients with/without associated thalassemia diagnosed with routinely employed laboratory parameters.

Materials and Methods

A retrospective study was conducted to assess the effects of HU on laboratory and clinical variables in SCD patients. The total study group consisted of 51 SCD patients who were randomly selected from those who attended the adult hematology clinic at Salmanya Medical Complex (SMC) which is a tertiary health care center in Bahrain. These cases were included on the basis of availability of clinical and laboratory investigation data of at least 1 year prior to as well as following the initiation of HU therapy. Ethical approval for the study was taken from the Health Research Committee, Ministry of Health, Bahrain.

Patient records were reviewed for documenting specific clinical and laboratory parameters during the 1 year preceding as well as 1 year following the date of initiation of HU treatment. While recording CBC data, it was verified that there was no incidence of blood transfusion during the period of 90 days preceding the test that could alter baseline values. Clinical variables that were recorded included: (a) number of hospital admissions; (b) total duration of stay in hospital in days; (c) total number of pain episodes; and (d) number of units of packed red cells administered during the period. Documented laboratory parameters included Hb, hematocrit (Hct), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), red cell distribution width (RDW) and hemoglobin fractions(HbF, HbS, HbA_2).

Hemoglobin (Hb) fractions were quantitated by high-performance liquid chromatography (HPLC) in the BIO-RAD Variant II system. All cases were screened for HbH inclusions in erythrocytes by supravital staining with brilliant cresyl blue. Patients were classified into four subgroups on the basis of laboratory test results and published criteria relating to identifying SCD associated with α- or β-thalassemia [2]. In addition, the cut-off level of HbA_2 (>4 %) that was used to discriminate sickle ß-thalassemia (S-β-thal) has been validated by the laboratory with molecular testing. These test groups and related classification criteria were as follows. Group 1, homozygous sickle cell anemia (SS, 24 cases): absence of microcytosis (MCV $\geq$ 80 fl) and HbA_2 less than 4 %. Group 2, SCD with microcytosis (SCD-m, 16 cases): low MCV (<80 fl) with no iron deficiency, HbA_2 level less than 4 % and HbH inclusion-negative. The rationale for separate grouping of these cases was a high likelihood of α-thalassemia association in these cases. Group 3, sickle α-thalassemia (SS-α-thal, seven cases), were diagnosed on the basis of presence of HbH inclusions in erythrocytes. Group 4, sickle ß-

thalassemia (S-β-thal, four cases): raised HbA_2 level (>4 %) and low MCV (<80 fl) supported by family study showing β-thalassemia trait in a family member (parents and/or siblings).

Data was analysed for statistical significance by SPSS software. Testing for differences between sub-groups was done by the Kruskal–Wallis test. The paired T test was used to test for significant differences between pre- and post-treatment variables within patient-groups. Pearson correlation coefficient was used to look for correlations between pre- and post-treatment variables.

Results

The total study group consisted of 27 males and 24 females. The majority of patients were young adults with a mean age of 29.6 ± 9.8 years. There was no significant difference between the mean ages in the four study groups. The dose of HU used in these patients was adjusted according to clinical and hematologic response and varied from 15 to 30 mg/kg/day.

Table 1 shows the results of laboratory tests in these patients before and after treatment with HU. The Hb levels ranged from 6.9 g/dl to 13.1 g/dl. When patients were grouped according to their Hb levels by WHO criteria [19], anemia was mild in 10 (Hb 11.0–12.9 g/dl), moderate in 37 (Hb 8.0–10.9) and severe in four cases (Hb < 8 g/dl). One patient was not anemic. 1 year following initiation of HU therapy the numbers of mild/moderate/severe anemia cases were 22/24/1 respectively. Four patients were non-anemic post-treatment. This included three patients in the SCA group whose Hb increased from mildly reduced to normal levels. In absolute terms, Hb increment was 0.6–1.1 g/dl in the different study groups with parallel increase of Hct. These changes in Hb level and/or Hct were highly significant in all with the exception of the S-β-thal group. There was no rise in the Hb level in ten cases. However, clinical indicators in six of these Hb non-responders showed improvement in all parameters whereas four showed reduction of severe crisis episodes (admissions, inpatient days) but not in the OPD attendance.

The HbF fractions in the study groups are shown in Table 1. Following treatment there was a rise in 39 (77 %) patients and the mean elevation was 4.5 % in the total study group. However there was improvement in *all* clinical parameters in 8/12 of the patients whose HbF levels did not increase. In the remaining four patients, a reduction in hospital admissions was noted in three and a total lack of improvement in one case only. The increment in HbF level was positively correlated with the magnitude of increase in Hb level ($P = 0.032$) and marginally so with increased MCH ($P = 0.057$). A highly significant inverse correlation between changes in HbF and HbS % was also observed.

In parallel with the changes in total Hb and HbF fraction, post treatment laboratory values in the total study group showed highly significant increases of Hct, MCV, MCH and RDW. When changes within the diagnostic sub-groups were examined, the changes in MCV were most consistent. The MCHC was slightly increased by the treatment in all groups except SS-α-thal but this was not statistically significant.

Table 2 shows the selected clinical parameters before and after HU therapy. There was a marked reduction in the numbers of hospital admissions, inpatient days, pain crisis episodes and the number of red cell units transfused in the total group as well as within the individual sub-groups. These changes were all highly significant with the exception of the group of four patients with S-β-thal in which despite marked reduction of all indices of clinical severity, only the reduction of pain crises was statistically significant.

When clinical parameters were correlated with the changes in laboratory variables, it was observed that there were significant correlations between increases in Hct and the reduction in the number of hospital admissions ($P = 0.04$) and the number of inpatient days ($P < 0.01$).

Discussion

After the FDA approval of HU therapy for the amelioration of SCD more than 15 years ago and with its widespread use, numerous reports have confirmed its beneficial effects. However, clinical responses, at least in some studies, have not been uniformly satisfactory [20]. Therefore studies have assessed HU response in different subsets of patients with SCD as part of a continuing search to identify factors that could explain this variability. Published reports have compared HU effects between groups of patients associated with different beta-globin gene haplotypes, interactions with other hemoglobin variants (such as HbC and HbO-Arab), various ethnic groups and genetic modifiers of HbF synthesis [10, 20–24].

The prevalence rates of sickle cell hemoglobinopathy and thalassemia are quite high in the Arabian Gulf region. In Bahrain, the reported rates according to different studies are: 13–16 % of sickle cell trait, 0.67–2.1 % of SCD, 2–2.9 % of β-thal trait and 24.3 % of α-thal trait [14]. The geographic distribution of the sickle cell gene co-exists with α^+-thal genotypes and this association is relatively common in the middle-east [25]. It is therefore surprising that there is no published data from this region that investigates whether this association with α-thal has any significant effect on the outcome of HU therapy in SCD.

Table 1 Laboratory parameters of sickle cell disease patients before and after hydroxyurea treatment

Parameter	SCA	SCD-m	SS-α-thal	S-β-thal	Total
Hb (g/dl)					
Before	10.0 ± 1.1	10.0 ± 1.6	9.9 ± 2.2	9.8 ± 1.1	10.0 ± 1.4
After	11.1 ± 1.0	10.5 ± 1.1	10.6 ± 1.2	10.9 ± 2.5	10.8 ± 1.2
	P < 0.0001*	P = 0.039	NS**	NS	P < 0.0001
Hct					
Before	0.30 ± 0.03	0.32 ± 0.04	0.30 ± 0.07	0.31 ± 0.03	0.31 ± 0.04
After	0.33 ± 0.03	0.33 ± 0.04	0.33 ± 0.04	0.35 ± 0.05	0.33 ± 0.04
	P < 0.0001	NS	P = 0.033	NS	P < 0.0001
MCV (fl)					
Before	87.9 ± 9.7	73.5 ± 6.0	76.2 ± 9.1	77.3 ± 11.6	80.9 ± 10.8
After	93.7 ± 9.3	81.2 ± 10	82.9 ± 12	86.0 ± 8.4	87.7 ± 11.2
	P = 0.002	P < 0.0001	P = 0.021	NS	P < 0.0001
MCH (pg)					
Before	29.3 ± 3.6	23.7 ± 2.2	24.9 ± 3.6	24.3 ± 4.7	26.5 ± 4.1
After	31.4 ± 3.3	26.4 ± 3.8	27.0 ± 3.8	27.8 ± 3.0	29.0 ± 4.1
	P = 0.001	P = 0.001	NS	NS	P < 0.0001
MCHC (g/dl)					
Before	33.2 ± 0.9	32.3 ± 0.8	32.6 ± 1.2	31.5 ± 1.2	32.7 ± 1.1
After	33.5 ± 0.9	32.5 ± 1.2	32.4 ± 0.7	32.3 ± 0.4	33.0 ± 1.1
	NS	NS	NS	NS	NS
RDW					
Before	17.9 ± 3.5	16.5 ± 2.2	22.4 ± 7.7	19.3 ± 1.1	18.2 ± 4.2
After	15.9 ± 2.3	16.0 ± 1.7	16.1 ± 2.9	17.5 ± 1.3	16.1 ± 2.1
	P = 0.009	NS	NS	NS	P = 0.001
HbF (%)					
Before	17.4 ± 6.0	14.5 ± 3.9	15.7 ± 3.3	11.7 ± 3.7	15.6 ± 5.1
After	21.3 ± 6.5	21.2 ± 8.6	19.3 ± 6.2	16.7 ± 2.8	20.1 ± 6.2
	P = 0.002	P = 0.004	NS	NS	P < 0.0001
HbS (%)					
Before	77.9 ± 6.4	79.7 ± 4.8	79.0 ± 3.9	79.6 ± 3.5	78.7 ± 5.4
After	71.4 ± 6.2	71.2 ± 5.6	70.2 ± 6.3	71.2 ± 2.7	67.5 ± 13.6
	P < 0.001	P = 0.002	P = 0.01	P = 0.004	P < 0.0001
HbA_2 (%)					
Before	2.2 ± 1.9	3.3 ± 1.6	3.1 ± 1.6	7.4 ± 0.2	3.1 ± 2.2
After	2.4 ± 1.6	3.5 ± 2.1	3.1 ± 1.5	5.5 ± 3.7	3.2 ± 2.1
	NS	NS	NS	NS	NS

* P values represent results of paired *t* test

** NS, not significant (P > 0.05)

Interestingly, in a study reported from London, Vasavda et al. reported that the increment of HbF by HU may not be as efficient in SS-α-thal as compared to SCA without associated α-thal [26]. The observed mean HbF increments in the two groups were 4.35 versus 8.24 % respectively. It was also observed that the reduction of inpatient days in the former was significantly smaller compared to the latter although the numbers of hospital admissions per year were not significantly different in the two groups. In our study, HU therapy led to significant HbF elevation in group 2 (probable SS-α-thal) and non-significant elevation in Group 3 (SS-α-thal). The non-significant elevation in the latter group may be due to the small number of cases analyzed. However, clinical improvement was significant in both groups in almost all parameters.

We did not observe any significant difference in the magnitude of HbF increase between SCD with or without associated α-thal. A study in India did not find any significant influence of α-thal on clinical scores in SCD patients treated with HU [24]. These observational

Table 2 Clinical variables reflecting disease severity in sickle cell disease patients before and after hydroxyurea treatment

Parameter	SCA	SCD-m	SS-α-thal	S-β-thal	Total
Admissions (no.)					
Before	2.6 ± 2.8	2.9 ± 3.0	2.9 ± 1.9	3.8 ± 3.0	2.8 ± 2.7
After	0.8 ± 1.6	0.5 ± 1.0	0.7 ± 1.3	0.5 ± 0.6	0.7 ± 1.3
	P < 0.0001	P = 0.001	P = 0.003	NS	P < 0.0001
Inpatient days					
Before	17.0 ± 16.0	20.5 ± 20.9	20.9 ± 13.9	23.0 ± 23.8	19.1 ± 17.6
After	4.9 ± 9.2	1.9 ± 3.5	3.9 ± 7.1	1.8 ± 2.1	3.6 ± 7.1
	P < 0.0001	P = 0.001	P = 0.008	NS	P < 0.0001
Pain crisis episodes					
Before	6.0 ± 6.6	17.9 ± 21.8	13.3 ± 8.3	25.5 ± 15.8	18.2 ± 17.5
After	2.2 ± 2.5	3.3 ± 3.6	4.7 ± 5.4	8.5 ± 10.4	5.2 ± 6.0
	P = 0.001	P = 0.019	P = 0.034	P = 0.016	P < 0.0001
Red cell transfusion (units)					
Before	2.3 ± 2.5	1.7 ± 2.3	2.1 ± 2.9	2.0 ± 2.2	2.0 ± 2.4
After	0.1 ± 0.4	0.2 ± 0.5	0	0	0.1 ± 0.4
	P < 0.0001	P = 0.018	NS	NS	P < 0.0001

Values represent observed numbers in one calendar year

NS Not significant

differences may be explained on the basis of other genetic associations such as the common occurrence of the Arab-Indian haplotype with the sickle cell gene in the Middle-East and in India that is associated with higher HbF levels in these patients. Sheehan et al. observed that the effects of HU therapy override genetic effects on the phenotype [13].

In the present study, SCD cases were divided into four sub-groups based on the probability of association of α- and/or β-thalassemia on the basis of routine screening tests. Identification of thalassemia association in these patients would be more specific with molecular studies but this is not generally undertaken in SCD in view of the cost of screening. Although this is one of the relative drawbacks of this study, for routine diagnostic purposes there is reliance on tests such as quantitation of Hb fractions by HPLC, staining for HbH inclusions in conjunction with evaluation of MCV, iron status and family studies [2]. Admittedly a few cases of α- or β-thalassemia may have been included in group 1 (SS) due to phenotypic overlap.

Institution of HU therapy led to remarkable improvement in the clinical status of almost all cases with the exception of one patient with SCA. In this male patient, there was no reduction in the number of pain episodes or hospital admissions however a transfusion requirement was eliminated. Clinical response was positively correlated with Hct and less so with Hb increments. However, changes in HbF levels correlated significantly with Hb and MCH changes. Despite obvious improvement in both laboratory and clinical parameters, the non-significant results in the group of four S-β-thal cases were likely the result of a small sample-size. In a similar analysis, Ware et al. reported that baseline Hb level, maximum tolerated dose and patient compliance correlated with the HbF response [27].

Our laboratory studies showed highly significant elevation of Hb, Hct, MCV, MCH and HbF % with reduction of RDW and HbS %. As proposed by Charache et al., higher MCV and MCH post-treatment may be due to increased Hb content of erythrocytes together with altered membrane properties and water content in these cells [5]. These hematologic changes have been amply observed previously although variations in the amplitudes of individual laboratory parameters occur across these reports. In a study that included 106 cases of SCA together with sickle-HbC (n = 7), S-β-thal (n = 7) and sickle-HbO-Arab (n = 2), HU therapy resulted in significantly increased Hb, MCV and HbF with reduced reticulocytes and bilirubin [23]. This suggests that HU therapy leads to a reduction of hemolysis in these cases. A review of 13 published reports including one randomized study on the results of HU treatment in adult SCD patients also reported higher Hb and HbF % with reduction of crisis rates and hospital admissions [28, 29]. The magnitude of elevation of Hb and HbF in our study (mean 0.8 g/dl and 5.4 % respectively) are similar to that reported in the literature. In published systematic reviews, mean elevation of Hb was 0.6 % and that of HbF was 3.2 % in adults [28, 29]. Children showed higher responses 1 and 10 % respectively [28]. Similar to the observations in our study, Italia et al. noted that clinical improvement was not correlated with the magnitude of HbF elevation indicating that other factors could also influence outcome [24]. Genetically, HbF levels are affected by several sis- and trans-acting factors and current studies

are focusing on non-ß-globin gene-associated genetic modifiers of HbF response.

In summary, treatment with HU showed excellent results in Bahraini SCD patients with or without associated α- or β-thalassemia. Therapy led to highly significant improvement in disease morbidity. Fetal Hb was variably elevated in all patient-groupswith consistently reduced HbS. The magnitude of these changes was not significantly correlated with the quantum of improvement of the evaluated clinical outcome parameters thereby suggesting the role of other factors. The results confirm that the effects of HU therapy in SCD override the influence of genetic modifiers as observed by Sheehan et al. [13].

Acknowledgments The study has been approved by the institutional research ethics committee, Ministry of Health, Kingdom of Bahrain and has been performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Conflict of interest Authors Durjoy K. Shome, Abdulla Al Ajmi, Ameera A. Radhi, Eman J. Mansoor and Kameela S. Majed declare that they have no conflict of interest.

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