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Hematological differences between patients with different subtypes of sickle cell disease on hydroxyurea treatment

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Objective: Sickle cell anemia and the interaction S/Beta thalassemia differ in hematological values due to microcytosis and hypochromia caused by the thalassemic mutation. The clinical benefit of long-term hydroxyurea treatment is undeniable in sickle cell disease with monitoring of the biological action of the drug being by the complete blood count. The objective of this work is to compare changes in some of the erythrocytic indexes between S/Beta thalassemia and sickle cell anemia patients on long-term hydroxyurea treatment.

Methods: The values of erythrocyte indexes (mean corpuscular volume and mean corpuscular hemoglobin) were compared in a retrospective study of two groups of patients (Sickle cell anemia and S/Beta thalassemia) on hydroxyurea treatment over a mean of six years.

Results: The quantitative values of the two parameters differed between the groups. Increases in mean corpuscular volume and reductions in mean corpuscular hemoglobin delay longer in S/Beta thalassemia patients (p -value = 0.018).

Conclusion: Hematological changes are some of the beneficial effects of hydroxyurea in sickle cell disease as cellular hydration increases and the hemoglobin S concentration is reduced. The complete blood count is the best test to monitor changes, but the interpretation of the results in S/Beta thalassemia should be different.

Keywords: Anemia, sickle cell; Hydroxyurea; Hemoglobinopathies; Erythrocyte indices

Introduction

Silvestroni and Bianco first described the S/Beta thalassemia association in Italy, in 1944⁽¹⁾. In Brazil, the first study on clinical, hematological and genetic characteristics of this association was performed in Ribeirão Preto by Zago et al.⁽²⁾. The hematological data provide elements to differentiate between S/Beta thalassemia and homozygous S (SS) as with S/Beta thalassemia, patients present microcytosis and hypochromia seen by the low values of mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), fetal hemoglobin increase (Hb F) and hemoglobin (Hb) A₂. The clinical progression is similar, although there are associations with benign mutations of thalassemia. The benefits resulting from treatment with hydroxyurea (HU) in patients with sickle cell disease are undeniable⁽³⁾ in particular for SS and S/Beta thalassemia individuals. Considering this, its use is highly recommended for patients with poor prognoses, which constitutes most affected individuals. Several studies⁽⁴⁻⁹⁾ have shown the benefits of HU in subjects with severe (β^0) or less severe (β^+) thalassemia mutations combined with Hb S. It remains to be proven whether HU can be extended to adult carriers of Hb SC, Hb SD associations and others; the results are favorable in children⁽¹⁰⁾.

Studies on S/Beta thalassemia are scarce due to the small number of patients and the reduced candidate group for HU treatment. The association with Beta thalassemia attenuates some aspects of the disease in some patients and an unfavorable clinical evolution, such as vaso-occlusive crisis (VOC), acute chest syndrome (ACS), several hospitalizations etc., is necessary to prescribe HU. Treatment using HU in S/Beta thalassemia was tested in Greek patients⁽⁴⁾ simultaneously with the Multicenter Study of HU in sickle cell anemia⁽³⁾. Most of the patients presented clinical improvement and increases in hematological values over the long term. In a later review, effects in children with S/Beta thalassemia⁽⁵⁾ were observed. Another study found premature hematological alterations (4 weeks)⁽⁶⁾, and in a more recent study⁽⁷⁾ the hematological response was significant after six months of HU treatment. In Sicily⁽⁸⁾, S/Beta thalassemia patients with poor evolution were studied and there was evident clinical improvement with increases in MCV but without increases in Hb levels and with few significant adverse effects. The largest prospective study on survival in S/Beta thalassemia patients⁽⁹⁾ showed a great reduction in clinical complications and increases in survival compared to patients who were not treated with HU, thus consolidating the significant benefits of the drug in this sickle cell syndrome. A prospective study on the action of HU in sickle cell anemia⁽¹¹⁾ showed early hematological alterations that constitute an easy manner to measure the action of the drug, thus electing the complete blood count as the best test to monitor toxicity and adherence.

Since 1999, sickle cell disease and HU treatment has been studied in Ribeirão Preto, Brazil. The different hematological values in homozygous S and S/Beta thalassemia patients led to a

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comparative study of HU response between these two groups, in particular in respect to MCV, which has been used to evaluate the biological action of HU and patient adherence to treatment.

Methods

A retrospective study was performed of 57 patients with sickle cell disease allocated to two groups: S/Beta⁰ and S/Beta⁺ thalassemia (S/Beta Group) and homozygous S (sickle cell anemia – SS Group). The diagnoses were made from the results of hemoglobin studies by alkaline pH electrophoresis and high-performance liquid chromatography and when possible by a familial study, characterizing either homozygous S (carriers of hemoglobin S with normal A₂ values) or S/Beta thalassemia (carriers of hemoglobin S with increased A₂ values). All patients took HU for a period of one to 11 years (mean six years) for several complications of the disease. The hematological indexes, i.e., total hemoglobin, total leukocyte count, neutrophils and platelets, MCV and the MCH concentration, were closely monitored. The values were checked each month and recorded in the third, sixth and 12th months during HU treatment and a current measurement (July, 2010) after 1 to 11 years. Regular doses of folate were administered and Vitamin B12 was dosed at least once, thus discarding the possibility of other causes of macrocytosis. Although several patients had sporadic erythrocyte transfusions during the study period, the recorded values were not influenced by the transfusions. All the SS and S/Beta⁰ thalassemia patients received prophylactic penicillin regularly. The Kruskal-Wallis test was used for the statistical analysis of the paired nonparametric values between the S/Beta thalassemia and SS groups and the Mann Whitney test was used to compare values between the third, sixth and 12th months and current values in relation to the basal measurements. Significance was set for a two-tailed p-value < 0.05. Total Hb and Hb F values, basal and after 12 months of HU

treatment were compared in both groups using the Wilcoxon test of the Prism 5 GraphPad program for Windows (GraphPad Software, Inc. 2012). The paired t-test (Excel v 12.1.1. 2008 for Mac) was used to analyze alterations in the MCV and MCH.

Results

The S/Beta Group was composed of 11 male and seven female patients with a mean age of 23.8 ± 8.6 years (range: 9-43 years old) and a mean HU dose of 24.7 ± 5.8 mg/kg/day. The means and standard deviations of Hb F varied from 5.5 ± 6.22% (range: 0.1-20.0%) before HU treatment to 21.1 ± 12.6% (range: 5.9-42.1%) after treatment (p-value = 0.0006) and total Hb ranged from 8.6 ± 1.4 g/dL (range: 6.1-11.3 g/dL) before to 9.5 ± 1.5 g/dL (range: 7.1-12.5 g/dL) after 12 months of treatment (p-value = 0.0075).

The SS Group had 19 male and 20 female patients with a mean age 26.7 ± 10.5 years (range: 11-59 years old) and average dose of HU 24.07 ± 5.5 mg/kg/day. The Hb F varied from 2.9 ± 2.1% (range: 0.5-8.4%) before HU treatment to 21.6 ± 7.4% (range: 7.4-34.5%) after treatment (p < 0.0001) and total hemoglobin varied from 8.4 ± 1.0 g/dL (range: 5.7-10.5 g/dL) before HU treatment to 9.2 ± 1.5 g/dL (range: 6.4-13.6 g/dL) after 12 months of treatment (p-value = 0.0023).

The indications for HU use were VOC, priapism, severe hemolysis, pulmonary hypertension and ACS for both the S/Beta and SS Groups, besides heart failure and ischemic stroke. The incidence of VOC and ACS was higher, albeit not significantly, in the SS group (t-test, data not shown).

The results for MCV and MCH concentrations of the groups in the third, sixth, and 12th months and after the follow-up (more than 1 to 11 years) are shown in Tables 1 & 2 as absolute values and increases (+) or decreases (-). The mean length of HU treatment was statistically similar for both groups.

Table 1 - MCV (mean ± standard deviation) and increases after hydroxyurea treatment in sickle cell disease patients

	S/Beta thalassemia		Hemoglobin SS		p-value
	MCV (fl)	% increase (*)	MCV (fl)	% increase (*)	
Basal	77.0 ± 10.5	0	91.8 ± 9.9	0	< 0.0001
3 months	90.6 ± 24.5	+17.6	104.9 ± 30.6	+14.2	0.013
6 months	96.5 ± 27.6	+25.3	105.9 ± 26.2	+15.3	0.0259
12 months	98.6 ± 16.4	+28.0	106.6 ± 28.0	+16.1	0.0538
Current	104.8 ± 14.0	+36.0	116.1 ± 14.1	+26.4	0.0020

*Percentage increase S/Beta thalassemia vs. SS – p-value = 0.018

Table 2 - MCH concentrations (mean ± standard deviation) and changes after hydroxyurea treatment in sickle cell patients

	S/Beta thalassemia		Hemoglobin SS		p-value
	MCH g/dL	% change *	MCH g/dL	% change *	
Basal	32.2 ± 2.3	0	33.2 ± 2.0	0	ns
3 months	31.9 ± 2.0	-0.9	33.5 ± 2.0	+0.6	0.0168
6 months	32.0 ± 1.8	-0.6	33.2 ± 1.8	0	ns
12 months	32.2 ± 1.9	0	32.9 ± 1.6	-1.2	ns
Current	30.7 ± 1.5	-4.6	32.1 ± 1.3	-3.6	0.0031

*Percentage change S/Beta thalassemia vs. SS – p-value not significant, ns: non-significant

Discussion

The hematological and cellular effects of HU treatment are expressed within the first weeks⁽¹¹⁾ with increases in total Hb, Hb F, MCH and MCV; in adults however, this is not always persistent during the entire treatment due to exhaustion of erythropoiesis. Once the medication is stopped, the hematological parameters return to the basal level after several months. The observed macrocytosis does not result from a lack of folate or vitamin B12, but from a rearrangement of the cellular protein synthesis. HU is an antimetabolite inhibitor of phase S (synthesis) of the cell cycle. Erythropoiesis occurs under tension due to the action of erythropoietin, which alters production to the fetal phase, producing gamma chains in the most primitive erythroblasts, which, along with alpha chains, produce Fetal Hb. More recently, the role of nitric oxide has been shown in hemoglobin synthesis where the guanosine monophosphate signaling system leads to the activation of the gamma-globulin gene (γ)^(12,13). The result is an increase in volume and cellular hydration, which reduces the concentration of hemoglobin S. In addition, S/Beta thalassemia has free alpha chains that conjugate with gamma chains thus producing more Hb F. According, for some authors, this is one of the reasons that HU treatment is better in S/Beta thalassemia compared to SS patients^(4,7,8).

In several studies there are overlapping results for SS and S/Beta thalassemia patients. Data referring only to S/Beta thalassemia are scarce. In 1995 Voskaridou⁽⁴⁾ studied 14 S/Beta thalassemia adults who had taken HU for 2 to 3 years. In 4 weeks they had presented great increases of Hb F, MCV and MCH, but this was lost over the long term. In the present study, as expected, patients presented increases in Hb F, total Hb and MCV in both groups, thus confirming the action of HU (with satisfactory adherence to treatment) in respect to sickle cell disease. Macrocytosis took longer to be expressed in S/Beta thalassemia due to microcytosis and hypochromia of the thalassemic cell population, which varies according to the beta gene mutation. The values of MCV in S/Beta thalassemia patients increased by 17.6% in three months, 25.3% in six months, 28% in 12 months and 36% after a mean follow-up of six years (p-value < 0.0001 in relation to the basal level). The values of MCV in SS individuals increased in the same periods by 14.2%, 15.3%, 16.1% and 26.4%, respectively (p-value < 0.0001). These results show that the increase in MCV was more intense in S/Beta thalassemia patients compared to SS individuals (p-value = 0.018). The average MCV in S/Beta thalassemia only exceeds 100 fl after a long time (after 12 months), whereas the MCV in SS subjects reaches this value within 3 months (Table 1). There was no significant difference between the groups in respect to the MCH concentrations (Table 2). Despite the increases in Hb F and total Hb, the MCH concentration did not change significantly. The greater effectiveness of HU in S/Beta thalassemia does not seem to be linked to the lower MCH concentration, which would mean a lower possibility of polymerization and, consequently sickling. In 1997, Rogers⁽⁵⁾, reporting on two children with S/Beta thalassemia being treated with doses of HU lower than the maximum tolerated, showed that the clinical improvement is similar to SS patients, as is the increase in Hb F and MCV. Koren⁽⁶⁾ studied 19 children and adults but did not separate S/Beta

thalassemia and SS patients. The study of Loukopoulos et al. of 55 S/Beta thalassemia and 14 SS Greeks stratified in SS, S/Beta⁰ thalassemia and S/Beta⁺ thalassemia Groups, showed increases in the total Hb and percentage of Hb F⁽⁷⁾. The authors found, at the end of the study with a maximum dose of 1.5 g/day HU, increases in total Hb in the SS and S/Beta⁰ thalassemia Groups, but not in the S/Beta⁺ thalassemia Group, and increases in the percentage of Hb F in all groups (p-value < 0.001). The data of the current study, without separating S/Beta⁺ thalassemia and S/Beta⁰ thalassemia, demonstrated increases in the total Hb and Hb F in both groups with HU doses similar to the study of Loukopoulos et al.

Loukopoulos et al. also found significant increases in MCV for all groups (26 fl - SS, 28.8 fl - S/Beta⁰ thalassemia and 20.8 fl - S/Beta⁺ thalassemia). The data of the current study differ from the study of Loukopoulos in respect to the MCH concentration, as there was a reduction, albeit not significant, in S/Beta⁰ thalassemia patients. The explanation might be the different degree of suspension of the beta globin synthesis with mutations present in the Greek⁽⁷⁾ (Caucasians) and Brazilian (mixed race) populations. The Brazilian population of S/Beta thalassemia might have benign African mutations^(2,14-17) besides the classical Mediterranean ones. In the Mediterranean region, two-thirds of the population with sickle cell disease is composed of S/Beta thalassemia with severe mutations in contrast to the Brazilian population with an absolute predominance of homozygous S. The study by Rigano⁽⁸⁾ of 22 S/Beta thalassemia individuals showed a significant increase (p-value < 0.001) in Hb F and MCV after HU treatment, which increased from 73.1 fl to 96.4 fl (+ 23.3 fl) by the end of the study. No differences were seen in the levels of total Hb and total leukocytes. This author as well as others^(4,7) emphasizes the better effect of HU in S/Beta thalassemia disease. The LaSHS study⁽⁹⁾, which focused on survival, did not separate the SS and S/Beta thalassemia Groups in respect to MCV, but confirmed the great benefit of the HU in 295 S/Beta thalassemia patients, with MCV levels increasing from 54% to 87% (S/Beta⁰) and from 66% to 82% (S/Beta⁺) after 10 years of continuous use. The limitations of the current study were that the mutations, the alpha:beta chain ratio and the presence of alpha thalassemia⁽¹⁸⁾, which may change the globular index were not identified, and other possible causes of macrocytosis, such as bone marrow dysplasia, were not investigated. Moreover, the study population was small, while in the other studies^(4,5,7,8), 93 S/Beta thalassemia patients were analyzed.

Conclusion

Macrocytosis resulting from the action of HU in S/Beta thalassemia patients measured by MCV, is lower than that observed in homozygous S at 6 months of treatment but it reaches a comparative value after more than 1 year. Microcytosis and hypochromia due to the thalassemia mutation contribute to this effect. The MCH concentration does not reduce significantly, but it is higher in S/Beta thalassemia. These differences must be considered when evaluating adherence to HU treatment and the hematological effects of this drug in S/Beta thalassemia. Multicenter studies with more patients are necessary to accurately evaluate the hematological alterations with HU treatment in the different subtypes of sickle cell disease.

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