Hydroxyurea alters hematological, biochemical and inflammatory biomarkers in Brazilian children with SCA: Investigating associations with βS haplotype and α-thalassemia

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Abstract

This study investigated the effects of hydroxyurea (HU) on hematological, biochemical and inflammatory parameters in children with sickle cell anemia (SCA) in association with β^S haplotype and α-thalassemia. We included 22 children with SCA who were followed for an average of 14.5 months. Laboratory parameters were assessed by electronic methods, and molecular analysis was investigated by PCR-RFLP and allele-specific PCR. Results showed significant increases in hemoglobin, HbF, hematocrit, MCV, MCH, glucose, HDL-C and albumin levels, as well as significant decreases in MCHC and AST levels, WBC, neutrophils, eosinophils, lymphocytes and reticulocytes, in children during HU therapy. HbF levels were positively correlated with hemoglobin, hematocrit, MCV and total protein, yet negatively correlated with MCHC, RDW, AAT and AST during HU therapy ($p < 0.05$). Children who carried the Central African Republic haplotype, in response to HU therapy, presented significant increases in hemoglobin, hematocrit, triglycerides and uric acid levels, as well as significant decreases in MCHC, AST and direct bilirubin levels, WBC, neutrophils, eosinophils, lymphocytes and reticulocytes. Those with the Benin haplotype presented increases in HbF and albumin levels, and a reduction in platelet counts ($p < 0.05$). Children with α-thalassemia presented decreased ALT during HU use, while those without this deletion presented increases in hemoglobin, hematocrit, MCV, MCH, HDL-C and albumin, as well as decreases in MCHC, neutrophils, lymphocytes, reticulocytes and AST ($p < 0.05$). Hence, regardless of its use in association with β^S haplotypes or α-thalassemia, HU seems to be linked to alterations in hemolytic, inflammatory, hepatic, lipid and glycemic profiles.

Introduction

Sickle cell anemia (SCA) is one of the most common inherited monogenic diseases in the world, characterized by chronic hemolytic anemia, vaso-occlusive events (VOE) and chronic organ injury [1]. Clinical profile and life expectancy vary widely among individuals with SCA, which can be explained by several factors, including genetic modifiers, such as haplotypes linked with the beta S (β^S)-globin gene cluster and alpha 2 deletion of 3.7 kb thalassemia ($\alpha^{2 \text{ del}}$ $_{3.7kb}$ thalassemia) [1]. The Benin (BEN), Bantu or Central African Republic (CAR), Senegal, Cameroon and Arab/Hindu haplotypes identified in the β^S-globin gene cluster have been associated with variability in fetal hemoglobin (HbF) levels, which is known to be a classic modulator of this disease [2–4]. Likewise, individuals with SCA who are carriers of α-thalassemia present increases in hemoglobin concentrations and red blood cell (RBC) count, as well as decreases in mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), reticulocyte counts and bilirubin levels [5–7]. However, α-thalassemia has also been associated with an elevated frequency of VOE, which may be due to increased hematocrit and, consequently, blood viscosity in individuals with SCA [7].

Among the three agents known to enhance HbF production (Sodium butyrate, 5-azacytidine and hydroxyurea—HU), HU, approved in 1998 by the U.S. Food and Drug Administration (FDA) for the treatment of individuals with clinically severe SCA, remains the most commonly used [3,8]. The main benefit associated with HU therapy is increased HbF levels, which have been directly associated with decreased HbS polymerization, reduced incidence of VOE, frequency and length of hospital stays, blood therapy and acute chest syndrome (ACS). HU therapy also reduces health care costs and increases survival rates [9–12]. Furthermore, studies have demonstrated the association of HU with a reduction in white blood cell (WBC) and reticulocyte counts, as well as bilirubin and lactate dehydrogenase (LDH) levels, in addition to increased MCV [3,4]. Although it is believed that increases in HbF levels mediates HU efficacy among individuals with SCA, it was reported that improvement in patients' clinical profiles appears prior to any significant increase in their HbF levels, suggesting that HU may modulate other laboratory parameters beside the classical increase seen in HbF [11]. Regarding HbF levels and the patient clinical profile, there is a great variability in response among individuals treated with HU, which may be due to genetic factors, including the β^S-globin gene cluster haplotype [13,14]. Moreover, despite evidence demonstrating its efficacy, HU is underused in younger individuals with SCA due to a range of issues, mainly side effects, which have not been completely elucidated [15,16].

Therefore, we sought to investigate the wider effect of HU on hematological, biochemical and inflammatory parameters in children with SCA who carry the β^S haplotype, with or without α-thalassemia, in a prospective study.

Materials and methods

Subjects and ethical aspects

The present prospective study included 22 children with SCA (HbSS). Laboratory parameters were assessed before and during HU treatment, and the median length of HU use was 14.5 months (6 to 72 months). The average age of the children was 8.5±3.4 years (median: 7.5 years) at the beginning of the study and 12 (54.55%) were female. All patients were seen at the outpatient service of the Fundação de Hematologia e Hemoterapia da Bahia (HEMOBA). They reported the prior regular use of folic acid, and, after study recruitment, HU was prescribed at doses lower than its maximum tolerated dose (MTD) and ranging between 10–25

mg/kg/day (median: 15 mg/kg/day). All legal guardians reported that HU treatment was very important to their children, and affirmed that the medication was taken regularly unless ordered to interrupt treatment by a physician, which strongly indicates HU compliance. For inclusion, children were required to be in steady state, characterized as the absence of acute crisis and without the use of blood therapy, for three months prior to blood collection procedures. Children on blood therapy and those with infections were excluded.

This study was approved by the Institutional Review Board of the São Rafael Hospital, and was conducted in accordance with the Declaration of Helsinki and its amendments. In addition, the legal guardians of all children signed a term of informed consent prior to enrollment in the study.

Laboratory methods

Blood samples were drawn at the time of each individual's enrollment by venipuncture, in the morning after 12 hours of fasting under standardized conditions. Hematological analyses were performed using a Cell Dyn-Ruby electronic cell counter (Abbott Diagnostics, Wiesbaden, Germany). Qualitative and quantitative hemoglobin profiles were determined by high-performance liquid chromatography (HPLC/Variant II; BIO-RAD, Hercules, CA, USA). Biochemical parameters were measured in serum by an immunochemistry assay using an A25 spectrophotometer analyzer (Biosystems SA, Barcelona, Spain). Inflammatory proteins, such as alpha-1 antitrypsin (AAT) and C-reactive protein (C-RP), were measured by immunochemistry using the Immage 800 System (Beckman Coulter, Fullerton, CA, USA). Serum ferritin was measured by immunoassay using an Access 2 Immunoassay system (Beckman Coulter, Fullerton, CA, USA).

Genomic DNA was extracted from peripheral blood using the Flexigen 250 kit (Qiagen, Hilden, Germany). β^S haplotypes were investigated by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) [17], and $\alpha^{2\ del\ 3.7kb}$ thalassemia was assessed by allele-specific PCR [18].

All analyses were performed at the Laboratório de Análises Clínicas and Laboratório de Pesquisa em Anemia, Faculdade de Farmácia, Universidade Federal da Bahia and the Laboratório de Investigação em Genética e Hematologia Translacional, Instituto Gonçalo Moniz–FIOCRUZ/BA.

Statistical analysis

Statistical analyses were performed using EpiInfo 7.0 and GraphPad Prism v5.0 software, with *p* values below 0.05 considered statistically significant. Distributions of quantitative variables were determined by the Shapiro-Wilk test. The mean values of variables (laboratory parameters), measured before and during HU therapy, were compared using the paired t-test for normal distribution, and Wilcoxon's test for non-normal distribution. Results were expressed as mean ± standard deviation. Pearson's correlation coefficient analysis was performed to assess the strength of linear relationships between two quantitative variables with normal distribution.

Results

Laboratory parameters of children with SCA before and during HU therapy

The laboratory parameters of the SCA children before and during HU therapy are shown in Table 1, and S1 Fig. After the onset of HU therapy, hematological analyses showed significant

Table 1. Laboratory parameters of SCA children, before and during HU therapy.

	Before HU, N = 22 M ± SD	During HU, N = 22 M ± SD	*p* value
Hemoglobin			
HbF, %	9.95±6.28	12.72±5.77	**0.04****
HbS, %	86.52±6.05	84.87±5.50	0.18*
Erythrogram			
RBC, x10^9/mL	2,74±0,63	2,74±0,57	0.89*
Hemoglobin, g/dL	8.15±1.30	8.77±1.24	**0.01***
Hematocrit, %	23.41±3.92	26.11±4.07	**<0.01***
MCV, fL	86.67±9.03	96.72±10.26	**<0.01***
MCH, pg	30.25±3.45	32.59±3.47	**<0.01***
MCHC, %	34.90±1.48	33.70±0.94	**<0.01***
Erythroblast (/10^2 leukocytes)	2.50±1.92	0.86±0.88	**<0.01****
Leukogram			
WBC, /mL	15283±3931	10772±3547	**<0.01***
Neutrophil, /mL	7275±2279	5174±2409	**<0.01***
Eosinophil, /mL	1097±816	508±439	**<0.01****
Lymphocyte, /mL	5717±2576	3958±1628	**<0.01***
Monocyte, mL	950±468	989±533	0.99**
Platelets			
Platelet, x10^3/mL	433±105	400±140	0.26*
Hemolysis			
Total bilirubin, mg/dL	2.54±1.77	2.69±1.65	0.30**
Direct bilirubin, mg/dL	0.54±0.29	0.43±0.24	0.13**
Indirect bilirubin, mg/dL	1.99±1.54	2.26±1.62	0.24**
Lactate dehydrogenase, U/L	1072±431.8	1026±375.3	0.51*
Reticulocyte, %	8.01±3.17	4.56±1.66	**<0.01***
Lipids and glucose			
Total cholesterol, mg/dL	120.0±26.0	124.7±29.6	0.28*
HDL-C, mg/dL	31.73±7.45	40.42±12.66	**<0.01****
LDL-C, mg/dL	69.96±19.69	64.35±23.62	0.20**
Triglycerides, mg/dL	92.23±51.44	106.5±55.65	0.05**
Glucose, mg/dL	71.00±9.35	83.86±9.58	**<0.01***
Renal panel			
Urea, mg/dL	18.86±7.48	21.04±8.85	0.43**
Creatinine, mg/dL	1.68±1.76	0.85±1.09	0.47**
Hepatic panel			
Aspartate aminotransferase, U/L	57.50±17.97	44.32±16.50	**<0.01***
Alanine aminotransferase, U/L	24.05±11.39	22.86±12.04	0.44**
Total protein, g/dL	7.84±0.70	8.17±0.77	0.23**
Albumin, g/dL	4.34±0.34	4.73±0.32	**<0.01***
Inflammatory markers			
Uric acid, mg/dL	2.48±1.72	3.41±1.77	0.07*
Ferritin, ng/dL	501.6±625.6	611.5±791.0	0.51**
C-reactive protein, mg/L	7.30±7.17	7.35±12.62	0.40**

(*Continued*)

Table 1. (Continued)

	Before HU, N = 22 M ± SD	During HU, N = 22 M ± SD	*p* value
Alpha 1 antitrypsin, mg/dL	168.3±39.98	104.2±47.41	**<0.01****

RBC: red blood cell, MCH: mean corpuscular hemoglobin, MCV: mean corpuscular volume, MCHC: mean corpuscular hemoglobin concentration, HbS: S hemoglobin, HbF: Fetal hemoglobin, WBC: white blood cell, HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol, VLDL-C: very low-density lipoprotein cholesterol, M ± SD: mean ± standard deviation, N: number of individuals

*Paired T-test

** Wilcoxon test

increases in mean HbF, hemoglobin, hematocrit, MCV and MCH, in addition to significant reductions in reticulocytes and MCHC ($p<0.05$). In all, 14 out of 22 children (63.64%) presented increases in HbF levels during HU therapy. Furthermore, all children had significant decreases in WBC, neutrophils, eosinophils and lymphocytes during HU therapy ($p<0.05$). Biochemical analysis revealed elevated glucose, HDL-C and albumin, as well as decreased aspartate aminotransferase (AST), in the children following the introduction of HU therapy ($p<0.05$).

## Correlations between HbF and laboratory parameters during HU therapy

Fig 1 shows the results of correlation analysis in SCA children undergoing HU therapy. HbF levels were found to be positively correlated with hemoglobin, hematocrit, MCV and total protein levels, yet negatively correlated with MCHC, RDW, AST and AAT ($p<0.05$). When performing correlation analysis in the children prior to the onset of HU therapy, no positive or negative associations were observed.

Laboratory parameters of children with SCA before and during HU therapy according to β^S haplotypes and α-thalassemia

The distribution analyses of β^S haplotypes and $\alpha^{2\ del\ 3.7kb}$ thalassemia showed that 45% were carriers of the CAR/CAR genotype, while 35% and 20% were CAR/BEN and BEN/BEN, respectively. Fourteen children (77.78%) presented the αα/αα genotype (without α-thalassemia) and 22.22% the -α/αα or -α/-α genotypes (with α-thalassemia).

As it is known that the response to HU can be affected by β^S haplotypes and α-thalassemia, we performed an association analysis using a dominant genetic model comparing all laboratory parameters of the children prior to and during HU therapy. This analysis showed that children with the CAR haplotype presented significant increases in hemoglobin, hematocrit, MCV, MCH, glucose, triglycerides, uric acid and albumin levels, as well as significant reductions in MCHC, AST, direct bilirubin and AAT levels, as well as WBC, neutrophils, eosinophils, lymphocytes and reticulocytes during HU therapy. Children who were carriers of the BEN haplotype presented elevated HbF, MCV, MCH, glucose and albumin levels, in addition to reduced platelets during HU therapy ($p<0.05$) (Table 2).

Regarding α-thalassemia, during HU therapy the children without α-thalassemia had elevated hemoglobin, hematocrit, MCV, MCH, glucose, HDL-C and albumin levels, in addition to decreased MCHC and AST, as well as WBC, neutrophils, eosinophils, lymphocytes and reticulocytes ($p<0.05$). Those with α-thalassemia presented significantly increased glucose levels and decreased WBC and eosinophils, as well as lower alanine aminotransferase (ALT) levels, during HU therapy ($p<0.05$) (Table 3).
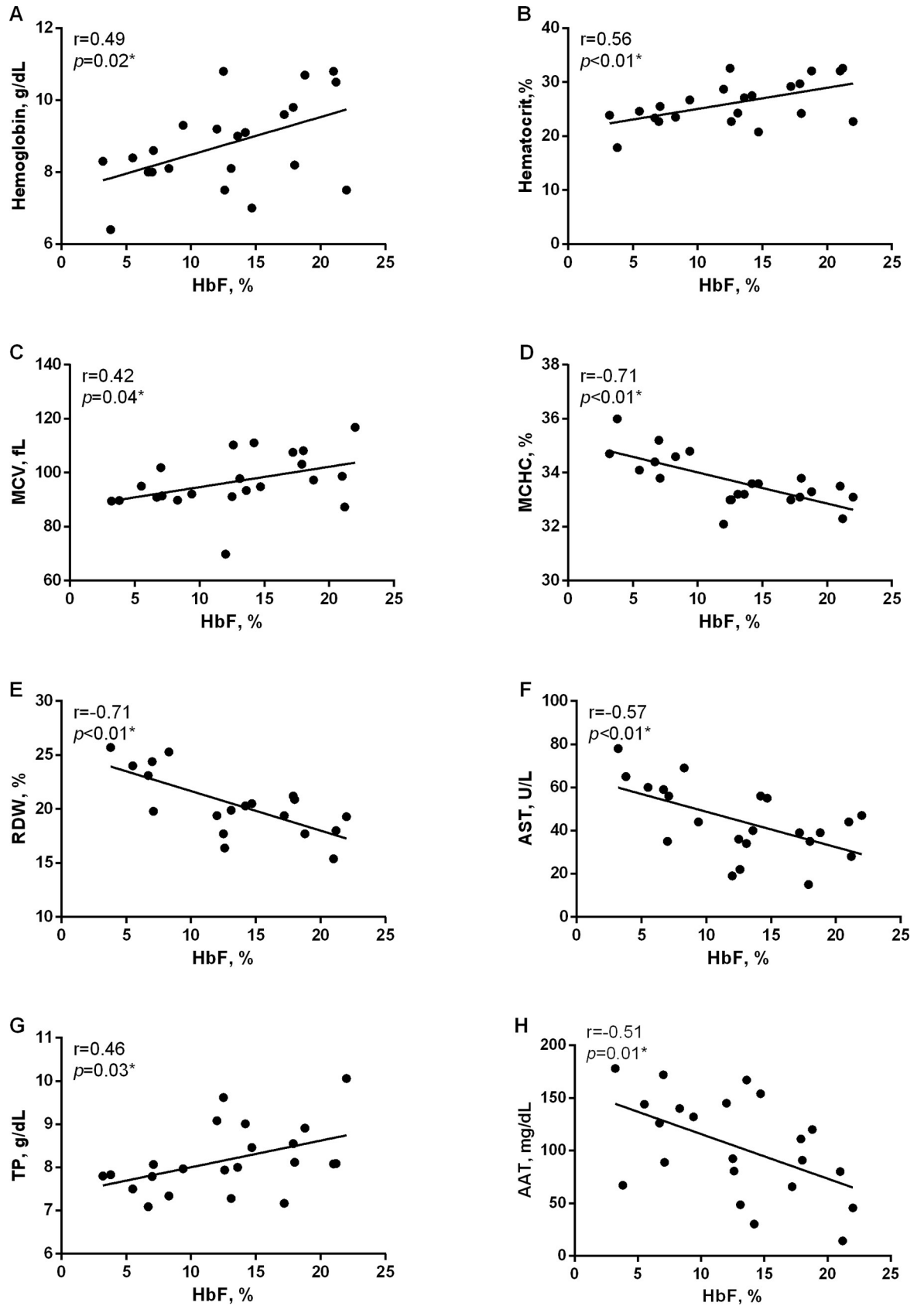
Fig 1. Correlations between HbF levels and hematological and biochemical parameters in children with SCA during HU therapy.
HbF levels were positively correlated with hemoglobin (**A**), hematocrit (**B**), MCV (**C**) and TP (**G**), while negatively correlated with MHCH (**D**), RDW (**E**), AST (**F**) and AAT (**H**). MCV: mean corpuscular volume, MCHC: mean corpuscular hemoglobin concentration, RDW: red cell distribution width, AST: aspartate aminotransferase, TP: total protein, AAT: alpha 1 antitrypsin, *Pearson's correlation.

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Discussion

The present study investigated the wider effect of HU on laboratory parameters in children with SCA, according to the presence of the β^S haplotype and α-thalassemia. It has been reported that individuals with SCA taking HU may present an improved clinical profile before significant increases in HbF levels are seen, and it is known that HU response can vary according to β^S haplotype and α-thalassemia [11,13,14].

As expected, HU intake resulted in a significant increase in HbF levels, which was observed in 14 (63.64%) children. This finding corroborates data from previous studies demonstrating that HU use was associated with higher HbF levels [19–22]. Moreover, increases in HbF were observed in some individuals [23–25], reinforcing the notion that not all individuals with SCA or SCD respond to HU therapy by initially presenting increased HbF levels. Additionally, HU was associated with higher hemoglobin, hematocrit, MCV and MCH, as well as lower MCHC and reticulocyte and erythroblast counts. These findings, also reported by previous studies [16,22,25–27], serve to confirm the association between HU therapy and an improvement in the hemolytic profile of individuals with SCA.

Table 2. Laboratory parameters of children with SCA according to β^S haplotype before and during HU therapy.

Parameter	CAR/CAR + CAR/BEN, n = 16			BEN/BEN, n = 4		
	Before HU	**During HU**	**_p_ value**	**Before HU**	**During HU**	**_p_ value**
HbF, %	10.57±6.68	12.14±5.46	0.27*	9.65±5.84	17.42±4.77	**<0.01***
Hemoglobin, g/dL	8.12±1.26	8.68±1.16	**0.03***	8.32±1.62	9.55±1.58	0.15*
Hematocrit, %	23.19±3.79	25.81±3.98	**<0.01***	24.13±5.38	28.73±4.63	0.07*
MCV, Fl	85.93±9.29	94.39±9.11	**<0.01***	87.30±10.32	104.45±11.62	**<0.01***
MCH, pg	30.13±3.39	31.90±3.29	**<0.01****	30.43±5.00	34.78±3.95	**<0.01***
MCHC, %	35.08±1.39	33.78±1.04	**<0.01***	34.68±1.88	33.30±0.29	0.50*
WBC, /mL	16004±3064	11149±2717	**<0.01***	10668±4978	6550±2646	0.14*
Neutrophils, /mL	7859±2064	5108±2035	**<0.01***	5161±2510	3288±1623	0.11*
Eosinophils, /mL	1186±836	600±459	**<0.01***	959±957	114±62	0.12**
Lymphocytes, /mL	5647±2181	4284±1653	**0.02***	3832±2049	2444±992	0.20*
Platelets, x10³/mL	427±87	430±131	0.92*	363±83	250±107	**0.03***
Reticulocytes, %	8.07±3.39	4.79±1.71	**<0.01***	7.02±3.10	3.75±0.46	0.25**
Glucose, mg/dL	71.06±9.05	84.50±7.92	**<0.01***	76.25±9.21	88.00±13.52	**0.01***
Triglycerides, mg/dL	92.94±57.96	116.2±62.59	**0.01***	101.8±33.08	84.25±11.44	0.31*
Uric acid, mg/dL	2.45±1.78	3.63±1.88	**0.03****	2.97±1.54	3.41±0.84	0.70*
AST, U/L	61.00±17.60	43.25±16.03	**<0.01***	49.00±17.61	45.75±8.26	0.58*
Direct bilirubin, mg/dL	0.58±0.31	0.40±0.19	**0.01****	0.37±0.22	0.44±0.18	0.28*
Albumin, g/dL	4.40±0.35	4.68±0.30	**<0.01***	4.32±0.30	5.0±0.36	**0.02***
Alpha 1 antitrypsin, mg/dL	165.9±43.19	111.6±45.23	**<0.01****	167±33.20	62.08±29.05	**0.01***

HbF: Fetal hemoglobin, MCH: mean corpuscular hemoglobin, MCV: mean corpuscular volume, MCHC: mean corpuscular hemoglobin concentration, WBC: white blood cell, AST: aspartate aminotransferase, n: number of individuals

*Paired T test

** Wilcoxon test, Data presented as means ± standard deviation

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Table 3. Laboratory parameters of children with SCA according to $\alpha^{2del\ 3.7\ kb}$ thalassemia before and during HU therapy.

	$\alpha\alpha/\alpha\alpha$, n = 14			$-\alpha/\alpha\alpha$ and $-\alpha/-\alpha$, n = 4		
Parameter	Before HU	During HU	*p* value	Before HU	During HU	*p* value
HbF, %	10.54±6.82	13.26±5.73	0.12*	8.61±5.91	9.57±3.10	0.71*
Hemoglobin, g/dL	7.81±1.13	8.52±1.17	**<0.01***	8.35±0.94	9.15±1.20	0.39*
Hematocrit, %	22.21±3.64	25.21±3.77	**<0.01***	24.53±2.66	27.55±4.01	0.30*
MCV, Fl	89.68±7.44	100.00±8.41	**<0.01***	77.13±10.24	85.84±10.69	0.07*
MCH, pg	31.65±3.03	33.87±2.47	**<0.01***	26.20±2.82	28.67±4.21	0.18*
MCHC, %	35.28±1.52	33.91±0.91	**<0.01****	34.05±1.35	33.32±0.99	0.54*
WBC, /Ml	15952±3534	10963±3091	**<0.01***	10300±3339	7675±2384	**0.04***
Neutrophils, /mL	7716±2103	4766±1956	**<0.01***	5061±1882	3792±1499	0.08*
Eosinophils, /mL	1250±961	607±484	**0.01****	1074±381	328±316	**<0.01***
Lymphocytes, /mL	5648±2196	4401±1770	**0.04***	3493±1134	2815±947	0.27*
Reticulocytes, %	7.75±2.78	4.51±1.27	**<0.01***	7.32±5.64	3.55±1.47	0.17*
Glucose, mg/dL	72±7.74	83.07±7.29	**<0.01****	75.50±8.58	91.50±14.20	**0.04***
HDL-C, mg/dL	31.14±7.94	37.50±9.39	**0.02****	30.50±6.56	52.30±21.95	0.18*
AST, U/L	61.00±15.97	45.57±14.43	**0.01***	51.50±25.25	42.50±18.70	0.24*
ALT, U/L	25.36±12.18	26.50±12.84	0.82**	26.75±12.50	16.25±6.24	**0.04***
Albumin, g/dL	4.30±0.33	4.69±0.30	**<0.01***	4.47±0.26	4.82±0.52	0.24*

HbF: Fetal hemoglobin, MCH: mean corpuscular hemoglobin, MCV: mean corpuscular volume, MCHC: mean corpuscular hemoglobin concentration, WBC: white blood cell, AST: aspartate aminotransferase, ALT: alanine aminotransferase, n: number of individuals

*Paired T test

** Wilcoxon test, Data presented as means ± standard deviation

The cytoreductive effect of HU, demonstrated by significant reductions in WBC, neutrophils, eosinophils and lymphocytes, is supported by other studies that also reported similar results [16,22,27,28]. In addition, we observed a significant decrease in levels of the anti-inflammatory protein AAT during HU therapy. These findings suggest an association between HU use and reduced WBC counts mediated and/or followed by decreases in AAT levels and, consequently, improvement in the inflammatory state generally presented by individuals with SCA [26].

In individuals with SCA, the anti-inflammatory role of HDL-C changes to a pro-inflammatory one when bound to free hemoglobin released during hemolysis; this has been associated with endothelial injury and worsening of the inflammatory profile [29,30]. In the present study, children also presented significant increases in HDL-C levels during HU therapy, with eight (36.36%) presenting HDL-C levels above reference values. This finding demonstrates the beneficial effect of HU on cholesterol, and corroborates data from our recent study showing an association between HU use and variation in cholesterol concentrations in individuals with SCA [3]. A previous study also showed that HU modulates the abnormalities of RBC membrane fatty acid composition, which in addition to the vasodilators, nitric oxide and Prostaglandin E2 generated by HU, may lead to clinical improvements prior to increases in HbF induced by HU therapy [31]. Surprisingly, the children also presented increases in glucose levels during HU therapy, although these remained within normal clinical ranges. This increase may be due to the changes observed in cholesterol concentrations in children during HU therapy, since it is known that glucose and lipid metabolism are linked to each other and that hypertriglyceridemia and low HDL-C may be the cause as well as the consequence of hyperglycemia [32]. In addition, significant decreases in AST levels and increased albumin were seen during the use of HU. Contrary to this finding, Colombatti and colleagues observed a

significant reduction in AST post-HU therapy [22], which may be due to differences in the clinical profiles of individuals in the respective study groups. Based on their findings, Ragg and colleagues suggested that, in addition to increased HbF observed in individuals with SCD treated by HU, some clinical improvements may be the result of a reduced imbalance in serum protein levels [33].

HbF levels were positively correlated with hemoglobin, hematocrit, MCV and total protein levels, in addition to negatively correlated with MCHC, RDW, as well as AST and AAT levels. A previous study demonstrated a negative correlation between HbF levels and hemolytic biomarkers, reticulocytes and LDH levels, in individuals with SCA [34]. Although we did not find this correlation, our results also suggest that the increases in HbF levels induced by HU are followed by improvements in the hemolytic and inflammatory profiles of the children with SCA studied herein. Furthermore, these findings suggest that, in addition to inducing HbF synthesis, HU can also alter metabolic and hepatic biomarkers by way of yet unknown direct or indirect mechanisms. A recent study performed in individuals with β-thalassemia demonstrated the effects of HU on the metabolic profile [35]. Previous studies also reported the effectiveness of HU for the treatment and prevention of proteinuria in individuals with SCD [36–38]. Nonetheless, further follow-up studies including larger numbers of individuals with SCA that evaluate HU dosage and length of use are needed to confirm the associations seen between HU and metabolic and hepatic biomarkers, as well as to investigate the clinical impact of these effects.

Regarding the distribution of haplotypes, 45% of the children were carriers of CAR/CAR genotype, while 35% and 20% had CAR/BEN and BEN/BEN genotypes, respectively, which corroborates previously reported frequencies in Brazilian individuals with SCA [13]. Our analysis of laboratory parameters, using the dominant genetic model, in children before and during HU therapy found significantly increased HbF levels only in carriers of the BEN haplotype on HU, which corroborates the results of a previous study [39]. These authors suggested that this may be explained by the high frequency of the favorable polymorphism *BCL11A* rs1427407 in these individuals. In the present study, HU was also associated with significant decreases in platelet counts in children with the BEN haplotype. In addition, children with the CAR haplotype presented significantly increased hemoglobin, hematocrit, triglycerides and uric acid, as well as significantly decreased MCHC, WBC, neutrophils, eosinophils, lymphocytes, reticulocytes, AST and direct bilirubin during HU therapy, which is suggestive of improvements in hemolytic, inflammatory and hepatic biomarkers. Regardless of haplotype, and in addition to being associated with significant increases in MCV and MCH, as well as significant decreases in AAT, HU therapy was also found to be associated with significantly increased levels of glucose and albumin.

Distribution analysis of $\alpha^{2\ del\ 3.7kb}$ thalassemia showed similar frequencies to those reported by Adorno and colleagues [40]. Using the dominant genetic model, laboratory parameter analysis in children before and during HU therapy revealed significantly decreased ALT during HU therapy in carriers of α-thalassemia, while those without α-thalassemia presented significant increases in hemoglobin, hematocrit, MCV, MCH, HDL-c and albumin, as well as significant decreases in MCHC, neutrophils, reticulocytes and AST. These findings corroborate previous studies suggesting that α-thalassemia attenuates the effect of HU [41–43], in addition to promoting a beneficial response to HU in SCA individuals without α-thalassemia. Contrary to our findings, other studies found significantly increased hemoglobin, hematocrit, MCV, MCH and HbF, as well as significantly decreased HbS, bilirubin and reticulocytes in SCA individuals with α-thalassemia on HU [25,44]. This discrepancy may be due to other intrinsic genetic factors in the individuals enrolled in the respective study groups. Our data further corroborate an association between HU and decreased WBC and eosinophils that was also

reported by a previous study [7]. However, we discovered increased glucose levels, regardless of the presence or absence of α-thalassemia.

Conclusion

The results of the present study confirm the association between HU therapy and higher HbF levels and suggest that exclusively focusing on HbF levels may not be the most suitable method of assessing HU response in children with SCA. Due to increases in HbF levels and/or via parallel pathways, HU therapy, whether in association or not with β^S haplotypes and α-thalassemia, is positively correlated with improvements in hemolytic and inflammatory profiles. Unexpectedly, our results also suggest that, at a dose under MTD, HU may also affect metabolic biomarkers, since it was found to be associated with changes in glucose, total protein, albumin, AST and HDL-C. The principal limitation of the present study is its relatively small sample size. Accordingly, the effects on metabolic biomarkers linked to HU therapy reported herein deserve further scrutiny in a larger study population.

Supporting information

S1 Fig. Laboratory parameters of SCA children, before and during HU therapy. All data presented statistical significant difference. MCH: mean corpuscular hemoglobin, MCV: mean corpuscular volume, MCHC: mean corpuscular hemoglobin concentration, HbF: Fetal hemoglobin, WBC: white blood cell, HDL-C: high-density lipoprotein cholesterol, AST: Aspartate aminotransferase, AAT: Alpha 1 antitrypsin.
(DOCX)

S1 File. Database.
(XLSX)

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