

## Analysis of oxidative status and biochemical parameters in adult patients with sickle cell anemia treated with hydroxyurea, Ceará, Brazil

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**Background:** Sickle cell anemia is a hemoglobinopathy caused by a mutation that results in the production of an abnormal hemoglobin molecule, hemoglobin S (Hb S). This is responsible for profound physiological changes, such as the sickling of red blood cells. Several studies have shown that hydroxyurea protects against vaso-occlusive crises.

**Objective:** The aim of this study was to evaluate the oxidative stress associated with biochemical parameters in patients with sickle cell anemia treated with hydroxyurea.

**Methods:** The study was conducted with 20 male and 25 female patients at the Hospital Universitário Walter Cantídio. The patients were divided into two groups: a study group (n = 12), patients with sickle cell anemia who were receiving hydroxyurea and a control group (n = 33) of sickle cell anemia patients not submitted to hydroxyurea treatment. The biochemical parameters analyzed were ferritin, transferrin, and serum iron. Glutathione was measured in its reduced form to analyze the oxidative state.

**Results:** The results showed insignificant increases in the levels of serum iron, transferrin and ferritin in patients treated with hydroxyurea when compared with those who did not take the medication. However, the glutathione levels were significantly higher in patients taking hydroxyurea than in controls.

**Conclusion:** These results indicate that hydroxyurea possibly acts as an antioxidant by increasing glutathione levels.

**Keywords:** Glutathione; Iron overload; Anemia, sickle cell; Reactive oxygen species; Hydroxyurea/therapeutic use

### Introduction

Sickle cell anemia (SCA) is the most common monogenic, inherited disease in the world. It originated in Africa and occurs predominantly in people of African descent. The disease has spread heterogeneously in Brazil because of racial miscegenation, thereby facilitating the continuity of this type of anemia in Brazil. It is considered a serious public health problem by Brazilian scientific literature.<sup>(1)</sup>

The disease is caused because of a point mutation at position 6 in the beta-globin gene that gives rise to an abnormal hemoglobin molecule called hemoglobin S (Hb S).<sup>(2)</sup> This causes physiological changes that affect the hemoglobin molecule in its deoxygenated state through the sickling of red blood cells; this triggers the formation of Hb S polymers, oxidative degradation of the Hb S molecule and the generation of oxidizing free radicals.<sup>(3,4)</sup> These erythrocytes have a greater adherence to the vascular endothelium, thus contributing to episodes of vaso-occlusion which is associated with the disease.<sup>(5,6)</sup>

Hb S is more unstable than normal Hb because the former releases high amounts of reactive oxygen species (ROS) ( $O_2^\bullet$ ,  $H_2O_2$ ,  $HO^\bullet$ )<sup>(7)</sup> and has reduced antioxidant capacity; this imbalance leads to oxidative stress. Previous studies have shown that the levels of glutathione, a major intracellular antioxidant, are lower in patients with SCA.<sup>(8,9)</sup> SCA patients require blood transfusions to improve oxygen transport and to improve blood volume. One of the complications of long-term transfusion therapy is iron overload.<sup>(10)</sup> Approximately 25% of the iron in the body of a normal adult is stored in the form of ferritin (each ferritin molecule has 4500 atoms of iron) and hemosiderin.<sup>(11)</sup> The main clinical manifestations of SCA are infections, acute chest syndrome (ACS), splenic sequestration, pain crises, renal disorders, cardiac disorders (heart failure), osteoarticular disorders (such as dactylitis or hand-foot syndrome), neurological disorders (stroke), ocular disorders, sores on the lower limbs and priapism.<sup>(12)</sup>

Several studies have shown that the use of hydroxyurea (HU) promotes higher levels of fetal hemoglobin (Hb F) in SCA patients and is useful in protecting against the sickling of red blood cells and vaso-occlusion. HU is a chemotherapeutic agent used to treat myeloproliferative disorders; it blocks DNA synthesis by interfering in the conversion of ribonucleotide to deoxyribonucleotide through the inhibition of ribonucleotide reductase and by keeping the cells in the S phase. HU began to be used in protocols to treat adult sickle cell disease patients in the 1980s.<sup>(13)</sup> This study aimed at evaluating the oxidative profile and biochemical parameters in SCA patients treated with HU and thus to assess the benefit of HU in improving the quality of life of patients.

## Methods

### Patient population

A cross-sectional, descriptive study was performed of 20 male and 25 female adult patients treated with HU for a period of 3 - 6 months (n = 12) or untreated (n = 33) as a follow-up activity at the outpatient clinic of the Hospital Universitário Walter Cantídio in Fortaleza between January and October 2009. This study was approved by the hospital's Research Ethics Committee.

### Laboratory methods

Peripheral blood (5 mL) was collected by venipuncture using heparin as an anticoagulant (to measure glutathione and total iron) and in a separator gel blood collection tube without anticoagulant (to measure ferritin and transferrin). The plasma was separated after centrifugation (10 min. at 1500 rpm) and washed with NaCl (0.9% w/v). Reduced glutathione (GSH) was quantified by spectrophotometry at 412 nm in heparinized plasma; the appearance of a yellowish color indicates a product of 5,5-dithiobis-2-nitrobenzoic acid (DTNB) oxidation.<sup>(14)</sup> Ferritin in the serum was measured by the microparticle enzyme immunoassay (MEIA) method developed by Abbott® using turbidimetry for the final quantification and is expressed in ng/mL. Transferrin was quantified in the serum by the colorimetric assay method using a Roche-Hitachi 917® analyzer; the results are expressed as mg/dL. To measure the total iron in the plasma (200 µL), nitric acid (4% v/v final concentration) was added to digest the sample. Digestion was performed at 200°C, until the volume was reduced to 1 mL. The iron was then released in its free form. The plasma iron content was determined using an atomic absorption spectrophotometer and an air-acetylene flame.<sup>(15)</sup>

### Statistical analysis

A descriptive analysis of the data was conducted using the GraphPad Prism 5 statistics program. The *t*-test was

used to analyze the association between variables. The level of significance was set at 5% (p-value < 0.05).

## Results

In this study, we evaluated glutathione levels and the iron profile of 45 sickle cell anemia patients (age range: 19 - 66 years; mean age: 31.4 years). The majority of the patients (65%) were mulattos followed by black patients (24%).

The mean serum levels of iron, transferrin and ferritin of the SCA patients treated with HU were  $106.0 \pm 15.0$  µg/dL,  $171.4 \pm 17.3$  mg/dL and  $473.2 \pm 87.4$  ng/mL, respectively and for the untreated patients (control) they were  $98.7 \pm 4.8$  µg/dL,  $162.8 \pm 13.8$  mg/dL and  $375.3 \pm 70.6$  ng/mL, respectively.

The average GSH levels in treated and untreated SCA patients were  $45.6 \pm 4.1$  µM and  $34.7 \pm 1.9$  µM, respectively. This difference was significant (p-value < 0.05).

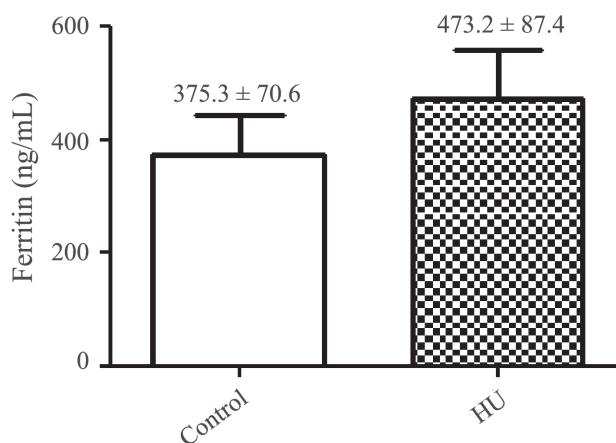


Figure 1 – Ferritin levels of patients taking hydroxyurea compared to controls (ng/mL)

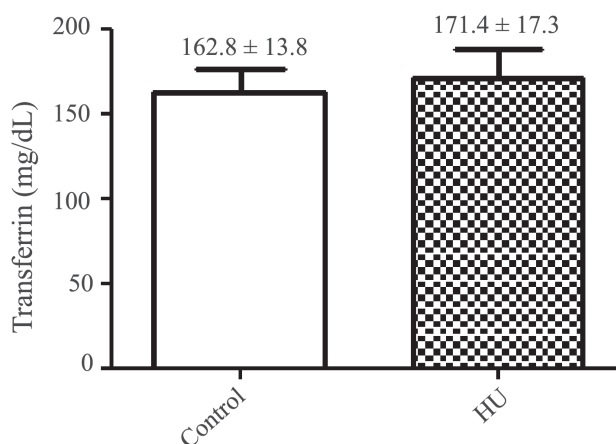


Figure 2 – Transferrin levels of patients taking hydroxyurea compared to controls (mg/dL)

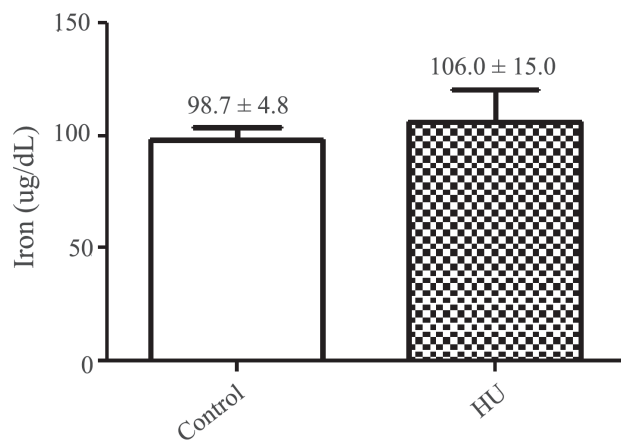


Figure 3 – Total iron levels of patients taking hydroxyurea compared to controls ( $\mu\text{g/dL}$ )

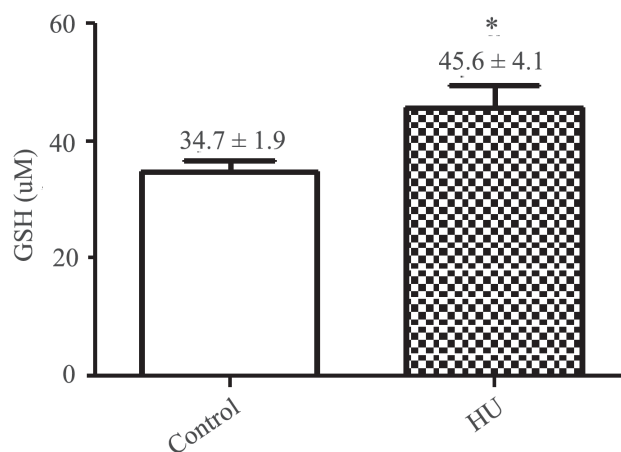


Figure 4 – GSH levels of patients taking hydroxyurea compared to controls ( $\mu\text{M}$ )

\*Data are reported as means ( $\pm$  SEM). \* $P < 0.05$  vs. control group (ANOVA and Student-Newman-Keuls Test)

## Discussion

Oxidative stress is a condition that results in an imbalance between the concentrations of pro- and anti-oxidant species. There is a strong correlation between the levels of glutathione in its reduced form (i.e. GSH) and enzymatic defense mechanisms. The resistance of many cells against oxidative stress is associated with high intracellular levels of GSH. One of the roles of GSH is to control the levels of lipid hydroperoxides and thus prevent cell damage by these radicals. Hence, glutathione levels may provide important biochemical information on the oxidant-antioxidant balance in the body.<sup>(9,16)</sup>

The results of the study show a significant difference in the GSH levels of SCA patients treated with HU and those who were not given this medication and are thus in line

with the results of another study,<sup>(17)</sup> in which the antioxidant effect of HU on the disease was demonstrated.

The results also showed an insignificant increase in the mean levels of iron, transferrin and ferritin in the serum of patients treated with HU when compared with those who did not take the medication. These results are similar to those reported by Manfredini<sup>(12)</sup> who demonstrated high levels of ferritin and serum iron and reduced levels of transferrin in SCA patients who did not take HU. The serum ferritin level is an indicator of iron stores and is considered the most important prognostic factor in patients with iron overload.<sup>(18)</sup> The accumulation of iron in the body is associated with the development and progression of various comorbidities with secondary iron overload being commonly found in SCA.<sup>(19)</sup> In terms of toxicity, chronic deposition of iron is often associated with the frequent blood transfusions required to treat certain types of anemia including SCA.<sup>(20,21)</sup>

The study showed that the use of HU may possibly act as a form of protection against oxidative stress by increasing the levels of GSH, the main intracellular antioxidant compound.

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