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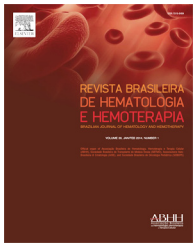

Original article

Pattern of hemolysis parameters and association with fetal hemoglobin in sickle cell anemia patients in steady state

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ABSTRACT

Objective: This study aimed to evaluate the influence of fetal hemoglobin (Hb F) on hemolysis biomarkers in sickle cell anemia patients.

Methods: Fifty adult sickle cell anemia patients were included in the study. All patients were taking hydroxyurea for at least six months and were followed at the outpatient clinic of a hospital in Fortaleza, Ceará, Brazil. The control group consisted of 20 hemoglobin AA individuals. The reticulocyte count was performed by an automated methodology, lactate dehydrogenase and uric acid were measured by spectrophotometry and arginase I by enzyme-linked immunosorbent assay (ELISA). The presence of Hb S was detected by high-performance liquid chromatography. The level of significance was set for a p-value <0.05.

Results: A significant increase was observed in the reticulocyte count and lactate dehydrogenase, uric acid and arginase I levels in sickle cell anemia patients compared to the control group (p-value <0.05). Patients having Hb F levels greater than 10% showed a significant decrease in the reticulocyte count, arginase I and lactate dehydrogenase. A significant decrease was observed in arginase I levels in patients taking hydroxyurea at a dose greater than 20 mg/kg/day.

Conclusion: The results of this study show that sickle cell anemia patients have increases in the hemolysis biomarkers, lactate dehydrogenase, reticulocyte count, arginase I, uric acid and increases in Hb F can reduce the reticulocyte count and arginase I and lactate dehydrogenase levels.

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Introduction

Sickle cell anemia (SCA) is an hemolytic anemia characterized by structural changes in the β-globin chain, leading to the synthesis of an abnormal hemoglobin (Hb) in homozygous (Hb SS).[1]

The primary event liable for any complications in SCA is the polymerization of Hb S, which culminates in hemolysis and vaso-occlusive events. These in turn trigger other events such as an inflammatory process, increased oxidative stress, endothelial dysfunction and decreased availability of nitric oxide (NO).[2]

Lactate dehydrogenase (LDH) is a marker of intravascular hemolysis and elevations in its plasma concentration are associated with the clinical phenotype of pulmonary hypertension, priapism and leg ulcers in SCA.[3] Studies have demonstrated that LDH may be a useful marker of disease complications related to hemolysis.[2,4]

Hyperuricemia occurs only in SCA patients who develop abnormal renal tubular function, with decreased uric acid clearance secondary to decreased urate excretion.[5]

Arginase is the enzyme that converts L-arginine to ornithine and urea. During the hemolysis process, this enzyme is released from red blood cells, contributing to the consumption of L-arginine and decreasing NO concentrations.[6,7]

There are two distinct isoforms of arginase (I and II), which are encoded by different genes and differ in molecular and immunological properties, tissue distribution, subcellular localization and regulation of expression.[8] Arginase I is primarily expressed in the liver and red blood cells, whereas the expression of arginase II (mitochondrial enzyme) is diffused in various tissues such as the brain, bone marrow, kidney, small intestine and mammary glands.[9] During the process of hemolysis, NO reacts with Hb to form methemoglobin and nitrate.

Arginase is an enzyme abundant in reticulocytes which predominate in patients with chronic hemolytic anemia, in particular SCA as there is a rapid turnover of red blood cells. Patients who have low arginine levels are more likely to develop pulmonary hypertension, stroke, priapism and leg ulcers, and consequently increased mortality.[2,7] Studies have shown a positive association of arginase I with hemolysis markers such as total bilirubin, indirect bilirubin and aspartate aminotransferase.[10,11]

Fetal hemoglobin (Hb F) is the most powerful modulator of the clinical and hematologic features of SCA influencing both clinical and laboratory features. The pathophysiology of this disease is dependent on the polymerization of deoxy-sickle Hb; the Hb F concentration reduces this process, thereby reducing hemolysis and vaso-occlusive events. The use of hydroxyurea may contribute to increased Hb F production, however, not all patients respond well to this drug.[12–15]

Thus, the present study aims to evaluate the impact of the Hb F on hemolysis biomarkers in adult patients with SCA.

Methods

Subjects and samples

This was a cross-sectional and analytical study of fifty adult SCA patients under outpatient treatment at the University Hospital of the Universidade Federal do Ceará (UFC) in Fortaleza, Ceará, Brazil from March 2012 to March 2013. All patients signed informed consent forms according to the research protocol approved by the Ethics Committee of the UFC. Eligibility criteria included adult patients with molecular diagnostics of SCA taking hydroxyurea at a dose of between 0.5 and 1.5 g/day for at least six months without recent blood transfusions. Transfusions were documented by the absence of Hb A measured by high-performance liquid chromatography (HPLC) (Ultra Resolution System, Trinity Biotech), according to the criteria described by Ballas et al.[16] A control group was composed by twenty blood donors (Hb AA).

Analysis of biomarkers

A venous blood sample (10 mL) was collected in a tube containing the ethylenediaminetetraacetic acid (EDTA) anticoagulant to manually perform a reticulocyte count and to measure the percentage of Hb F by HPLC analysis. Moreover a 6-mL venous blood sample was collected in a tube with separator gel but without anticoagulant to measure serum LDH, uric acid and arginase I. LDH and uric acid were measured by kinetic analysis using the Labtest® kit. The serum concentration of arginase I was determined according to the enzyme-linked immunosorbent assay (ELISA) kit for human arginase protocol (USCNK Life Science Inc.). The kit is a sandwich enzyme immunoassay for *in vitro* quantitative measurement of serum arginase I.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 5.0 (USA). Initially, data normality was analyzed using the Kolmogorov–Smirnov test. The unpaired *t*-test and Mann–Whitney test were used to compare two numerical variables. A multiple comparison of means was performed using the analysis of variance (ANOVA) test (Bartlett's test for equal variances) followed by Newman–Keuls post-test to identify which groups were different. The Spearman test was used to correlate the arginase I with Hb F concentration, reticulocyte count, LDH and uric acid levels. All results were expressed as means ± standard error of the mean (SEM). The level of significance for all analyses was set for a p-value <0.05.

Results

The demographic and hematological characteristics of the patients are shown in Table 1. Significant increases in the reticulocyte count, LDH, uric acid and arginase I were observed in patients with SCA compared to the control group (p-value <0.05) (Table 2). Patients with Hb F concentration lower than