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To the editor:

Lactate dehydrogenase isoenzyme 3 and hemolysis in sickle cell anemia: a possible correlation?

The concept that lactate dehydrogenase (LDH) is a useful clinical marker of hemolysis in sickle cell anemia (SCA) is still a subject of debate.[1,2] Some previous data suggested that the increase in serum LDH in these patients was mainly due to tissue damage rather than hemolysis.[1,2] However, other authors had shown a clear correlation between LDH and plasma hemoglobin (Hb).[3,4] The 5 LDH isoenzymes are separable by electrophoretic mobility and have different tissue origins: LDH-1 and LDH-2 are found in erythrocytes and cardiac muscle; LDH-3 in lymphatic tissue, platelets, lung, and the pancreas; and LDH-4 and LDH-5 in the liver and skeletal muscle.[5] Recently, a strong correlation was demonstrated between LDH and erythrocyte-derived microvesicles, a product of hemolyzing erythrocytes.[4,6] Nevertheless, there is scarce information about these isoenzymes in SCA; therefore we evaluated LDH and its isoenzymes in the hemolytic process of SCA.

We tested 40 consecutive SCA patients, with a median age of 30 years (P25-P75: 22 to 37), 28 (70%) women, who were assigned to 2 groups: sickle cell (SS) (20 patients without hydroxyurea [HU])

Table 1. Characteristics of the groups of patients with SS, patients in use of HU, and CG

	SS (n)	SS-HU (n)	CG (n)
Age (y)	30.5 (21.2-46.2) (20)	29.5 (24-37) (20)	31 (27-39.2) (22)
Gender (M/F)	8/12	4/16	9/13
Hemoglobin (g/dL)	9.2 (7.9-10) (20)	9.5 (8.4-10.1) (20)	14.1 (13.7-15.2)* (19)
Mean corpuscular volume (fL)	86.8 (78.2-96) (20)	111 (106-116.3)† (20)	90.1 (86.2-92.1) (19)
Lactate dehydrogenase (U/L)	526 (384-691) (20)	351.5 (280.5-425.5) (20)	152 (136-172)* (19)
LDH-1 (U/L)	225.7 (161.6-318.1) (20)	154.9 (115.7-180.8) (19)	31 (27.5-37.2)* (16)
LDH-2 (U/L)	203.2 (146-268.7) (20)	132.5 (102-159.8) (19)	60.5 (52.9-70.2)* (16)
LDH-3 (U/L)	56 (38.2-70.1)‡ (20)	33.7 (31.7-40.8) (19)	28.5 (25.3-34.3) (16)
LDH-4 (U/L)	20.3 (14.9-24.9) (20)	17.5 (14.2-18.7) (19)	17.3 (12.7-20.5) (16)
LDH-5 (U/L)	15.1 (11.7-19.8) (20)	12.9 (11.3-17.1) (19)	16.5 (14.3-23.9) (16)
Plasma hemoglobin (μg/dcL)	104.6 (44.5-223.8) (20)	51.2 (30.1-77.6) (20)	14.4 (10.3-30.4)* (22)
Haptoglobin (μg/dL)	0.1 (0.0-0.3) (20)	0.6 (0.1-1.6) (20)	116.7 (75.9-151.2)* (21)
Microvesicles (μL)	32 185 (17 059-53 980) (20)	21 643 (9942-41 673) (20)	5595 (3886-8691)* (20)

Results are expressed as median (P25-P75). Kruskal–Wallis with Dunn's Multiple Comparison Test.
*CG vs SS and SS-HU ($P < .0001$).
†SS-HU vs SS and CG ($P < .0001$).
‡SS vs SS-HU and CG ($P < .0001$). All other parameters showed no significance.

and SS-HU (20 patients who were using HU at the maximum tolerated dose of 27.4 mg/kg, P25-P75: 23.2 to 28.8). Twenty-two healthy individuals composed the control group (CG) with a median age of 31 years (P25-P75: 27 to 39), 13 (59%) female. Patients with painful crisis or red blood cell transfusion in the preceding 3 months, and pregnant women were excluded. Study approval was obtained from the Federal University of São Paulo, the institutional review board for these studies. Informed consent was provided according to the Declaration of Helsinki. Complete blood cell count (Cell-Dyn 370, Abbott Diagnostics) and serum level of LDH were obtained by routine methods (Cobas, Roche); LDH isoenzymes by electrophoresis on agarose gel and specific enzymatic revelations (Interlab G26); plasma-Hb determination by spectrophotometry; erythrocyte-derived microvesicles by flow cytometry (FACSCalibur, BD Biosciences)[7]; and haptoglobin by immunoturbidimetry (Olympus AU 640 system). Kruskal–Wallis and Spearman correlation tests were performed using GraphPad Prism 5 (GraphPad Software Inc.). A P value of $<.05$ was considered significant.

Laboratory data comparing the 3 groups are described in Table 1 and the main differences observed were: (1) high mean corpuscular volume; and (2) low LDH-3 in SS-HU compared with the SS group. As expected, LDH, LDH-1, and LDH-2 were elevated in both groups of patients.[8] Because the great majority of the hemolytic parameters did not present statistical difference between the SS and SS-HU groups (Table 1), they were considered as one group to perform the correlation analysis. These tests showed positive correlations: LDH ($r = 0.693$), LDH-1 ($r = 0.683$), LDH-2 ($r = 0.739$), LDH-3 ($r = 0.644$), LDH-4 ($r = 0.590$) × plasma-Hb; and LDH ($r = 0.581$), LDH-1 ($r = 0.609$), LDH-2 ($r = 0.596$), LDH-3 ($r = 0.419$) × microvesicles; all $P < .001$. An inverse correlation was also observed between haptoglobin and LDH ($r = -0.499$), LDH-1 ($r = -0.530$), LDH-2 ($r = -0.514$), LDH-3 ($r = -0.365$); all $P < .03$. No other correlations involving LDH-4 and LDH-5 were observed.

HU, the only drug treatment currently approved for SCA, has many benefits on SCA, such as increase in Hb and fetal Hb, reduction of white blood cells and platelets, lower adhesiveness of sickled red cells to endothelium, and decrease of reticulocytes and LDH.[9,10] Although previous data suggested hemolysis-lowering effects of HU characterized by reduced reticulocytes and LDH, our results did not show a reduction of LDH or other hemolytic parameters, in patients who use HU.[9] However, we observed an important and unexpected decrease in LDH-3 in the SS-HU group. Because LDH-3 has been previously associated with lung injury,[1,5] we performed statistical analysis with data of pulmonary artery pressure obtained by echocardiogram and observed no correlation with LDH-3 levels (data not shown). Furthermore, there was no difference in LDH-3 levels between SS-HU and the CG, which draws attention to a possible role of this isoenzyme in hemolysis or even in the HU treatment follow up. Our data showed a strong correlation of LDH, LDH-1, and LDH-2 with hemolysis (plasma-Hb, microvesicles, and haptoglobin), which was previously documented with total LDH.[3,4,6] These results are in accordance with others studies that had already demonstrated increased serum levels of LDH-1 and LDH-2 in SCA, confirming that these isoenzymes are the dominant source of serum LDH in this disease.[3,4] To the best of our knowledge, this is the first report to show a positive correlation between LDH-3 and hemolytic parameters in SCA that needs to be further clarified. A possible role of this isoenzyme as a biomarker of HU treatment also remains to be determined.

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Contribution: G.M. collected the samples, performed the research, and wrote the manuscript; M.S.F. and M.Y. designed the research study; and M.S.F., M.Y., and T.P.B. revised the paper critically.

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