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Evaluation of Ischemia-Modified Albumin in Anemia Associated to Chronic Kidney Disease

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Chronic kidney disease (CKD) is highly prevalent, with increasing numbers of patients affected by the disease world-wide, and anemia is a common finding in patients with CKD. Anemia impacts negatively on cardiovascular disease, exercise capacity, and quality of life, resulting in significant mortality and morbidity. The aim of this study was to evaluate the levels of ischemia-modified albumin and lactate in patients with established anemia associated with CKD and its correlations with hemoglobin levels. Hematocrit, hemoglobin, iron, ferritin, albumin, creatinine, lactate, and ischemia-modified albumin (IMA) were measured in 17 patients with established anemia associated to CKD and 19 controls by standard methods. The results of hematocrit, hemoglobin, iron, and albumin were lower in the anemia group than in the control group. Ferritin, creatinine, and lactate levels were higher in anemia of the CKD group than the control group. IMA increase in the anemia group (0.8115 ± 0.1304 absorbance units [ABSU]) compared to control (0.4951 ± 0.0393 ABSU). Significant correlations between IMA and lactate, IMA and hemoglobin, IMA and creatinine, and hemoglobin and lactate were observed. IMA and lactate increase during anemia and this elevation could be associated to hypoxia due to low hemoglobin levels. However, our data suggest that lactate is more sensitive to anemia compared to IMA. J. Clin. Lab. Anal. 22:1–5, 2008.

Key words: ischemia-modified albumin; lactate; hemoglobin; anemia; ischemia

INTRODUCTION

Chronic kidney disease (CKD) is highly prevalent, with increasing numbers of patients affected by the disease world-wide (1). Anemia is a common complication that contributes to the burden of disease associated with CKD, and it impacts negatively on cardiovascular disease, exercise capacity, and quality of life, resulting in significant mortality and morbidity in patients with CKD (2–5). Anemia is currently defined by the World Health Organization (WHO) as a hemoglobin (Hb) level < 13 g/dL in men and < 12 g/dL in women (6). Iron-deficiency anemia (IDA) is the most common anemia and it affects an estimated 1–2 billion people worldwide. In developing countries, over 50% of pregnant women are anemic, as are 46–66% of children under 4 years old, with one-half attributed to iron deficiency (7,8).

Human serum albumin, a single chain of 585 amino acids, consists of three structurally homologous, largely helical domains (I, II, and III), and each domain consists of two subdomains, A and B (9). The first three amino acids in the N-terminus, Asp-Ala-His, is a specific

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binding site for transition metals such as cobalt (II), copper (II), and nickel (II), and the most susceptible region for degradation compared with other regions of albumin. Ischemia, hypoxia, acidosis, and free radical formation can transiently alter the ability of the residues to bind free metal atoms (10–13). On the basis of these biochemical changes, Bar-Or et al. (13) described a rapid colorimetric assay method measuring ischemia-induced alterations of the binding capacity of human serum albumin to exogenous cobalt. Ischemia-modified albumin (IMA) has been shown to be a rapidly rising and sensitive biochemical marker especially for the diagnosis of myocardial ischemia (13–15). Ceyhan et al. (16) reported a negative linear correlation between lactate levels and hemoglobin values in anemic children, and hypoxia associated with anemia could be responsible for the higher levels of lactate. Therefore, we reasoned that IMA and lactate measurement could be useful for the evaluation of hypoxia associated to lower levels of hemoglobin in anemia. The aim of this study was to evaluate the levels of IMA and lactate in patients with established anemia associated to CKD and its correlations with hemoglobin levels.

MATERIALS AND METHODS

We investigated 17 patients submitted to hemodialysis with established anemia associated to CKD from Caridade and Casa de Saúde Hospitals, located in Santa Maria, RS, Brazil. A total of 11 were men, and six were women, and their age was 51.6 ± 12.8 years (range, 29–77 years). Patients with alcoholism, smoking, diabetes, viral hepatitis, and HIV were excluded from this study. A total of 19 healthy subjects were included in the control group. Of the 19 subjects, 10 were men, and nine were women, and their age was 43.7 ± 5.4 years (range, 37–55 years). All patients gave written informed consent, and this study protocol was approved by the institutional ethics committee (number 0109.0.243.000–06). Blood samples were collected by venous puncture into purple top, gray top, or red top Vacutainer® (BD Diagnostics, Plymouth, UK) tubes containing ethylene diamine tetraacetic acid (EDTA), sodium fluoride and potassium oxalate, or no anticoagulant, respectively. Specimens were routinely centrifuged within 1 hr of collection for 15 min at 1,000 *g*, and aliquots of serum samples were stored at −20°C for a maximum of 4 weeks before IMA measurement.

Hematocrit and hemoglobin were measured in whole blood collected in EDTA tubes by use of standard methods with the fully automated PENTRA 120® (ABX Diagnostics, Montpellier, France) system. Plasma lactate and serum levels of iron and creatinine were measured by use of standard methods with the fully automated VITROS 950® (Ortho-Clinical Diagnostics, Rochester, NY) dry-chemistry system. The serum levels of albumin were measured by the bromcresol green method in Cobas MIRA® Plus (Roche Diagnostics, Basel, Switzerland) analyzer, and serum ferritin was measured by use of a chemiluminescence immunoassay in the IMMULITE 2000® (Diagnostics Products Corporation, Los Angeles, CA) system.

Serum IMA was measured by the albumin cobalt binding test on a Cobas MIRA® Plus analyzer according to the method described by Fagan et al. (12) and validated in previous studies (12,17). A total of 95 μL of patient serum is pipetted to the reaction cuvette on the Cobas MIRA® Plus analyzer. A total of 5 μL of a 16.8 mM $CoCl_2$ solution chased with 20 μL of barbital buffer (pH 8.6) is added 25 sec later. The sample/cobalt/buffer mixture is incubated for 275 sec to allow binding of cobalt to albumin then a blank read optical measurement is made at 500 nm. A total of 25 μL of 9.7 mM dithiothreitol (DTT) is added 25 sec later. DTT reacts with unbound (non-N-terminal sequestered) cobalt to form a colored product. The final reaction mixture is incubated for an additional 100 sec and read at 500 nm. All incubations are at 37°C. Total assay time once the sample in pipetted is 7.5 min. IMA results were expressed in absorbance units (ABSU) as described previously (13,15).

Data were expressed as mean ± standard error (SEM). The Mann-Whitney test was used to evaluate the difference between groups. Spearman rank correlation was used to evaluate the associations between IMA, lactate, hemoglobin, and creatinine, and $P < 0.05$ was considered statically significant.

RESULTS

We evaluated 36 patients in this study, and the levels of hematocrit, hemoglobin, iron, and albumin were lower in anemia of the CKD group than the control group. Ferritin, creatinine, and lactate levels were higher in the anemia group than the control group, as shown in Table 1. The levels of IMA were elevated in anemia associated with the CKD group (0.8115 ± 0.1304 ABSU) compared to control group (0.4951 ± 0.0393 ABSU), as indicated in Fig. 1. We also observed significant correlations between IMA and hemoglobin ($r = -0.3442$, $P = 0.0398$), IMA and lactate ($r = 0.4616$, $P = 0.0046$), IMA and creatinine ($r = 0.5008$, $P = 0.0019$), and lactate and hemoglobin ($r = -0.7195$, $P < 0.0001$), as shown in Fig. 2.

DISCUSSION

The results of the present study indicate that IMA levels and lactate concentrations increase in patients

TABLE 1. Characteristics of study participants[†]

	Control	Anemia
Hematocrit (%)	42.68 ± 1.27	30.19 ± 1.24***
Hemoglobin (g/dL)	14.43 ± 0.54	9.77 ± 0.40***
Iron (μg/mL)	71.95 ± 3.95	53.82 ± 3.90*
Ferritin (ng/mL)	83.79 ± 6.92	213.60 ± 38.13**
Albumin (g/dL)	4.00 ± 0.06	3.17 ± 0.04***
Creatinine (mg/dL)	0.86 ± 0.04	5.52 ± 0.37***
Lactate (mmol/L)	1.07 ± 0.05	2.75 ± 0.29***

[†]Values are given as mean ± SEM.
*$P < 0.05$.
**$P < 0.01$.
***$P < 0.001$.

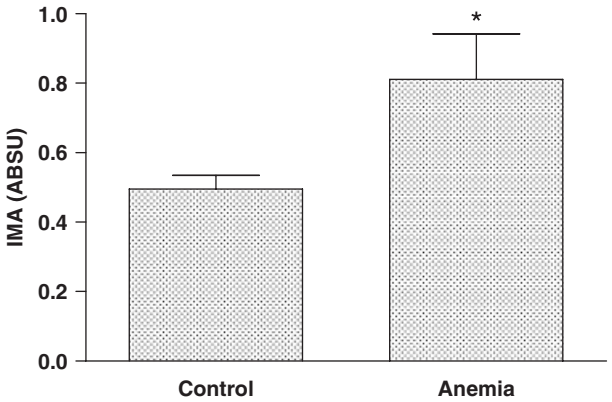

Fig. 1. IMA values observed in the study participants. *$P < 0.05$.

with anemia associated with CKD. Sharma et al. (18) recently reported that IMA level predicts mortality in patients with end-stage renal disease, and patients with elevated IMA levels have larger left ventricular size, decreased systolic function, and greater estimated left ventricular filling pressures. Ischemia may alter the metal binding capacity of circulating serum albumin, and biochemical mechanisms involved in the in vivo alterations to metal-albumin binding during either ischemia or reperfusion may include hypoxia, acidosis, free radical damage, membrane energy-dependent sodium and calcium pump disruptions, and free iron and copper ion exposure (13,19,20). The first three amino acids in the N-terminus, Asp-Ala-His, are a specific binding site for transition metals and the most susceptible region for degradation compared with other regions of albumin (10–13). However, Mothes and Faller (21) recently suggested that the first two equivalents of Co^{II} bind to sites A and B, and only the third may be bound to the N-terminal site. They also speculate that the structural changes in human serum albumin and subsequent lower levels of Co^{II} binding in myocardial ischemia could be linked to fatty acid binding.

Renal failure is associated with kidney function loss with the increase of serum creatinine, and it is accompanied by oxidative stress, which consists of the damage of biological structures by reactive oxygen species (ROS) due to their excessive generation and impaired efficiency of antioxidant defense mechanisms (22). The increase of IMA levels in patients with anemia associated with CKD could be attributed to the increase of oxidative stress normally observed in patients with chronic renal failure, and also to the decrease of albumin levels observed and the resultant increase in the nonbound portion of cobalt. Generation of ROS can transiently modify the N-terminal region of albumin and produce an increase in IMA levels (23,24). Some authors (24,25) reported that ROS generation in vitro causes structural changes in a synthetic N-terminus tetrapeptide, an octapeptide, and human albumin with loss of Co^{2+} binding capacity. High or low albumin concentrations may affect IMA testing, producing lower or higher values, respectively, even within the reference interval. Some authors (26,27) recently reported formulas for the adjustment of IMA by serum albumin, but these were not still validated in any way for CKD patients. The increase of IMA levels in anemic patients could also be attributed to mild hypoxia due to low hemoglobin levels, and this hypoxia is responsible for the alteration in metal-albumin binding during anemia. The reduction of hemoglobin levels could change the tissue oxygen delivery. There are also reports suggesting a role for hemoglobin-induced variations in arterial O_2 content (28,29).

Our results confirm previous studies that reported the increase of lactate levels in anemia (16,30–32). Lactate is the end product of anaerobic glycolysis and high blood lactate concentration can indicate tissue hypoxia in trauma or septic, hypovolemic, or cardiogenic shock. Anemia has been implicated in the decreased oxygen tension. The high lactate dehydrogenase-5 levels found in anemic patients are certainly in accordance with the switch-on of anaerobic metabolism, presumably a result of reduced oxygenation offered by the low hemoglobin levels (33). Lactate and hemoglobin showed the best relationship in this study, with a significant negative correlation. The inverse correlation between lactate and hemoglobin levels in anemia was previously reported, and the hypoxia caused by anemia could be responsible for the higher levels of lactate (16,30). Ohira et al. (32) showed that lactate levels were significantly elevated in whole blood and plasma from iron-deficient anemic rats, and IDA induces an elevation of lactate production following an increase in total lactate dehydrogenase (LDH) activity and change in LDH isoenzyme patterns.

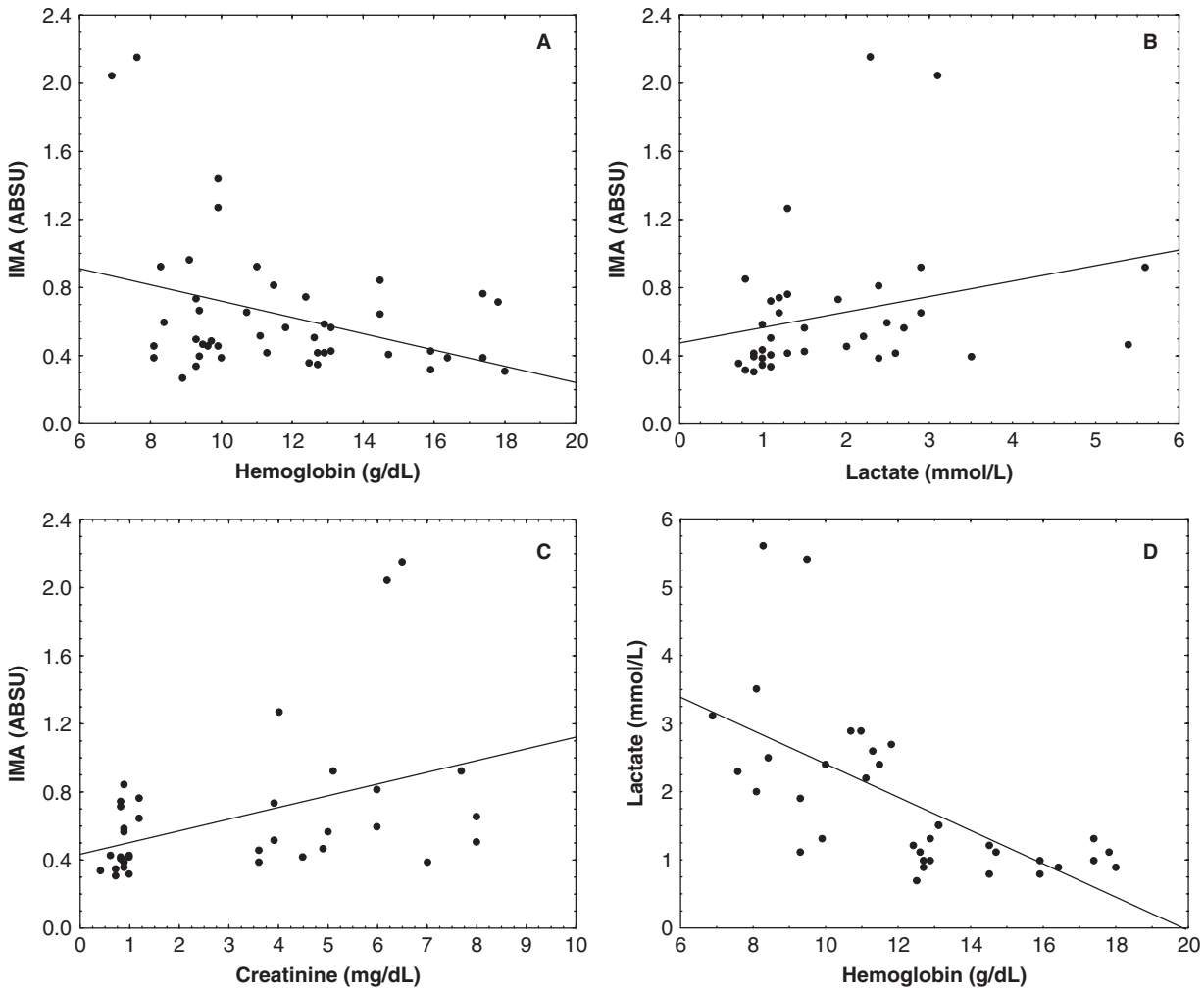

Fig. 2. Significant correlations between (**A**) IMA and hemoglobin ($r = -0.3442$, $P = 0.0398$), (**B**) IMA and lactate ($r = 0.4616$, $P = 0.0046$), (**C**) IMA and creatinine ($r = 0.5008$, $P = 0.0019$), and (**D**) lactate and hemoglobin ($r = -0.7195$, $P < 0.0001$).

In summary, we have shown that IMA and lactate increase during anemia and this elevation could be associated with hypoxia due to low hemoglobin levels. Although lactate is an insensitive marker of ischemia, our data indicate that it is more sensitive to anemia compared to IMA. However, further epidemiological studies are required to understand the mechanisms leading to increase of IMA and lactate levels in anemia and other different clinical conditions associated to ischemia, and also to evaluate IMA value as a tool in the diagnosis of diseases.

REFERENCES

1. El Nahas AM, Bello AK. Chronic kidney disease: the global challenge. Lancet 2005;365:331–340.
2. Hsu CY. Epidemiology of anemia associated with chronic renal insufficiency. Curr Opin Nephrol Hypertens 2002;11:337–341.
3. Kerr PG. Renal anaemia: recent developments, innovative approaches and future directions for improved management. Nephrology 2006;11:542–548.
4. Perlman RL, Finkelstein FO, Liu L, et al. Quality of life in chronic kidney disease (CKD): a cross-sectional analysis in the Renal Research Institute CKD study. Am J Kidney Dis 2005;45:658–666.
5. Eschbach JW, Adamson JW. Anemia of end-stage renal disease (ESRD). Kidney Int 1985;28:1–5.
6. Eisenstaedt R, Penninx BW, Woodman RC. Anemia in the elderly: current understanding and emerging concepts. Blood Rev 2006;20:213–226.
7. Lozoff B, Georgieff MK. Iron deficiency and brain development. Semin Pediatr Neurol 2006;13:158–165.
8. Stoltzfus RJ. Iron deficiency: global prevalence and consequences. Food Nutr Bull 2003;24(4 Suppl):S99–S103.
9. He XM, Carter DC. Atomic structure and chemistry of human serum albumin. Nature 1992;358:209–215.
10. Cho DK, Choi JO, Kim SH, et al. Ischemia-modified albumin is a highly sensitive serum marker of transient myocardial ischemia induced by coronary vasospasm. Coron Artery Dis 2007; 18:83–87.

11. Sbarouni E, Georgiadou P, Theodorakis GN, Kremastinos DT. Ischemia-modified albumin in relation to exercise stress testing. J Am Coll Cardiol 2006;48:2482–2484.
12. Fagan GJ, Wayment H, Morris DL, Crosby PA. The albumin cobalt binding test: analytical performance of a new automated chemistry assay for the detection of ischemia modified albumin (IMA). Journal of Clinical Ligand Assay 2002;25:178–187. http://www.ingentaconnect.com/content/clas/jcla
13. Bar-Or D, Lau E, Winkler JV. A novel assay for cobalt-albumin binding and its potential as a marker for myocardial ischemia: a preliminary report. J Emerg Med 2000;19:311–315.
14. Sinha MK, Roy D, Gaze DC, Collinson PO, Kaski JC. Role of "ischemia modified albumin", a new biochemical marker of myocardial ischaemia, in the early diagnosis of acute coronary syndromes. Emerg Med J 2004;21:29–34.
15. Bhagavan NV, Lai EM, Rios PA, et al. Evaluation of human serum albumin cobalt binding assay for the assessment of myocardial ischemia and myocardial infarction. Clin Chem 2003;49:581–585.
16. Ceyhan M, Ozalp I, Altay C. High levels of lactate, pyruvate, and alanine in anemic children. Clin Pediatr 1988;27:206–209.
17. Gidenne S, Ceppa F, Fontan E, Perrier F, Burnat P. Analytical performance of the albumin cobalt binding (ACB®) test on the Cobas MIRA® Plus analyzer. Clin Chem Lab Med 2004; 42:455–461.
18. Sharma R, Gaze DC, Pellerin D, et al. Ischemia-modified albumin predicts mortality in ESRD. Am J Kidney Dis 2006;47:493–502.
19. Christenson RH, Duh SH, Sanhai WR, et al. Characteristics of an albumin cobalt binding test for assessment of acute coronary syndrome patients: a multicenter study. Clin Chem 2001; 47:464–470.
20. Berenshtein E, Mayer B, Goldberg C, Kitrossky N, Chevion M. Patterns of mobilization of copper and iron following myocardial ischemia: possible predictive criteria for tissue injury. J Mol Cell Cardiol 1997;29:3025–3034.
21. Mothes E, Faller P. Evidence that the principal Co^{II}-binding site in human serum albumin is not at the N-terminus: implication on the albumin cobalt binding test for detecting myocardial ischemia. Biochemistry 2007;46:2267–2274.
22. Valentini J, Schmitt GC, Grotto D, et al. Human erythrocyte delta-aminolevulinate dehydratase activity and oxidative stress in hemodialysis patients. Clin Biochem 2007;40:591–594.
23. Roy D, Kaski JC. Ischemia-modified albumin: the importance of oxidative stress. J Am Coll Cardiol 2007;49:2375–2376.
24. Roy D, Quiles J, Gaze DC, Collinson P, Kaski JC, Baxter GF. Role of reactive oxygen species on the formation of the novel diagnostic marker ischaemia modified albumin. Heart 2006; 92:113–114.
25. Chan B, Dodsworth N, Woodrow J, Tucker A, Harris R. Site-specific N-terminal auto-degradation of human serum albumin. Eur J Biochem 1995;227:524–528.
26. Lippi G, Montagnana M, Salvagno GL, Guidi GC. Standardization of ischemia-modified albumin testing: adjustment for serum albumin. Clin Chem Lab Med 2007;45:261–262.
27. Lee YW, Kim HJ, Cho YH, Shin HB, Choi TY, Lee YK. Application of albumin-adjusted ischemia modified albumin index as an early screening marker for acute coronary syndrome. Clin Chim Acta 2007;384:24–27.
28. Roach RC, Koskolou MD, Calbet JA, Saltin B. Arterial O_2 content and tension in regulation of cardiac output and leg blood flow during exercise in humans. Am J Physiol 1999; 276:H438–H445.
29. Saltin B, Kiens B, Savard G, Pedersen PK. Role of haemoglobin and capillarization for oxygen delivery and extraction in muscular exercise. Acta Physiol Scand 1986;128:21–32.
30. Haaland K, Kofstad J, Apricena F, Thoresen M. Haemoglobin is inversely related to plasma lactate and heart rate in the newborn piglet. Biol Neonate 1996;69:350–356.
31. Gregg SG, Mazzeo RS, Budinger TF, Brooks GA. Acute anemia increases lactate production and decreases clearance during exercise. J Appl Physiol 1989;67:756–764.
32. Ohira Y, Chen CS, Hegenauer J, Saltman P. Adaptations of lactate metabolism in iron-deficient rats. Proc Soc Exp Biol Med 1983;173:213–216.
33. Koukourakis MI, Giatromanolaki A, Polychronidis A, et al. Endogenous markers of hypoxia/anaerobic metabolism and anemia in primary colorectal cancer. Cancer Sci 2006;97:582–588.