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Case Report

Pseudo-anion gap acidosis

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Case

A 74-year-old Caucasian male was referred to our clinic for low serum bicarbonate levels and a high anion gap (HAG). Past medical history included hypertension, hypothyroidism, iron deficiency anaemia secondary to gastrointestinal bleeding and hyperlipidaemia. He was on Bicitra 30 mL orally three times daily, levothyroxine 75 mcg daily, atorvastatin 10 mg daily, ferrous sulphate 324 mg daily and chlorthalidone 12.5 mg daily. He denied smoking, alcoholism or illegal drug abuse. Physical exam was unremarkable.

His laboratory values obtained over the 18 months after our evaluation are outlined in Table 1. Arterial blood gas showed a pH of 7.37,P_{CO_2} of 35 mmHg andP_{O_2} of 87 mmHg when the serumHCO_3^- level was 12 mEq/L. Serum lactate levels on several occasions were $<$2.2 mmol/L. Serum ketones were negative and the beta-hydroxybutyrate level was mildly elevated at 3.60 mg/dL (normal 0–3.0 mg/dL). Salicylate level was $<$1 mg/dL. D-Lactate levels were immeasurable. Thiamine levels were normal. A serum and urine protein electrophoresis did not reveal any paraproteinaemia. Urinalysis revealed a specific gravity of 1.020, pH 6.0 with negative protein and blood by dipstick. Urine toxicology screens for ethanol, methanol, ethylene glycol and isopropanol were negative. His urine organic acid profile was normal and no 5-oxoproline was detected. He was started on various forms of bicarbonate supplementation and atorvastatin was stopped, but neither bicarbonate levels nor anion gap changed significantly (Table 1).

In the absence of any apparent explanation for a decrease in serum bicarbonate with an HAG despite extensive work-up, we questioned the validity of the laboratory testing.

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As such, venous blood was drawn from the patient into a heparinized tube, with equal aliquots taken for enzymatic measurement of bicarbonate [1] and indirect measurement utilizing reference electrode assessment of pH and carbon dioxide, followed by logarithmic calculation of the bicarbonate level [2]. TheHCO_3^- level by the former was 10 mEq/L. Blood gas technology provided a venous HCO_3^- measurement of 31 mEq/L with a pH of 7.43,P_{O_2} of 78 mmHg andP_{CO_2} of 46 mmHg. Multiple repeat measurements confirmed this finding.

To assess the possibility of an endogenous inhibitor or interfering substance, a sample of the patient's serum (7.7 mEq/L bicarbonate by an enzymatic method) was sequentially diluted with either a normal serum (25.6 mEq/L bicarbonate) or a commercial bicarbonate solution (31.9 mEq/L). Bicarbonate values were determined by the enzymatic method and compared to the predicted content of bicarbonate in the admixtures. Recovery of bicarbonate was at least 85–90% at all dilutions (Figure 1). This does not support the presence of an excess soluble inhibitor, since the patient's serum did not significantly affect detection of bicarbonate in admixtures. Conversely, recoveries did not rise above 100%, indicating that suppression of the patient's bicarbonate measurement was not due to a weak or low concentration inhibitor that could be overcome with dilution.

It has been reported that the calculated bicarbonate method has a positive bias relative to the enzymatic method [3,4]. To determine the magnitude of this bias in our laboratory, 88 pairs of patient results for both calculated and enzymatic bicarbonate were compared. As shown in Figure 2, a positive bias of 1.7 mEq/L was observed. This is in close agreement with a positive bias of 1.6 mEq/L that was reported by Story *et al.* [3]. While a positive bias in the calculated bicarbonate is observed, it is very unlikely that our patient's observed difference of 21 mEq/L between the calculated and enzymatic methods is explained by this methodologic bias.

The underlying source of HAG acidosis is usually detected based on the clinical scenario and the basic laboratory test results [5]. Apart from the common causes of metabolic acidosis, several cases of HAG acidosis due to rhabdomyolysis, hyperphosphataemia, toluene poisoning, thiamine deficiency, statins, D-lactic acidosis, accumulation

Table 1. Laboratory results of our patient over 18 months

Variables	Normal values	Dates						
		January 2006	March 2006	July 2006	November 2006	January 2007	March 2007	June 2007
Na (mmol/L)	132–146	141	140	142	140	140	140	138
K (mmol/L)	3.5–5.5	4.4	4.4	4.3	4.6	4.1	4.3	4.9
Cl (mmol/L)	99–109	111	106	106	104	106	106	106
HCO_3 (mmol/L)	20–32	10	13	12	10	<10	10	<10
Anion gap	7–16	20	22	24	26	>24	24	>22
Glucose (mg/dL)	74–106	94	85	92	94	89	82	86
BUN (mg/dL)	9–23	29	20	29	32	26	20	29
Cr (mg/dL)	0.7–1.3	1.5	1.4	1.3	1.4	1.6	1.3	1.3
Phos (mg/dL)	2.4–5.1	2.8	3.2	2.8	3.2			2.8
UA (mg/dL)	3.7–9.2				9.0	7.6		
Albumin (g/dL)	3.2–4.8	4.1	4.3		4.5	4.1	4.0	4.3
Mg (mEq/L)	1.1–2.2				1.9	1.9		
Ca (mg/dL)	8.6–10.2	8.1	8.6	8.6		8.8	8.9	9.1
Hb (g/dL)	14–17.5	8.8	11.9	12.7	11.6	10.6	11.9	13.6
Hct (%)	42–52	27	36	38	34	33	36	42

Na = sodium, K = potassium, Cl = chloride, HCO_3 = bicarbonate, BUN = blood urea nitrogen, Cr = creatinine, Phos = phosphorus, UA = uric acid, Mg = magnesium, Ca = calcium, Hb = haemoglobin, Hct = haematocrit.

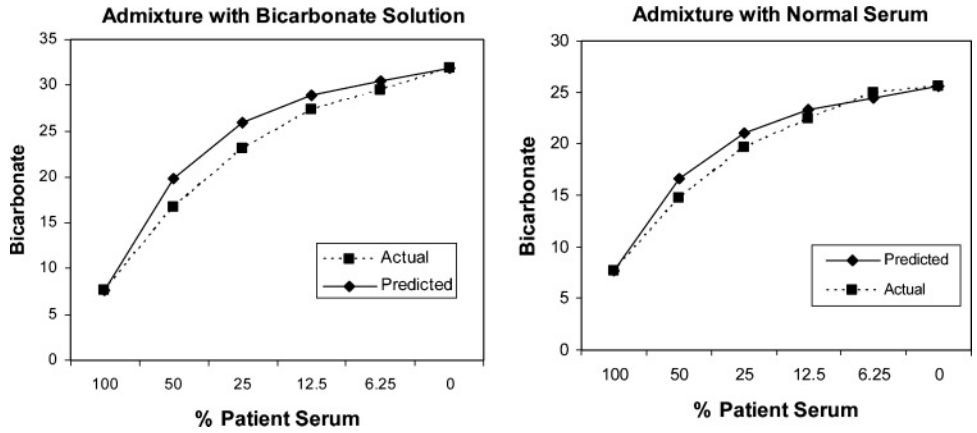

Fig. 1. Admixtures of the affected patient serum with other bicarbonate sources yield predicted bicarbonate levels. Aliquots of affected serum (7.7 mEq/L) were mixed with either a bicarbonate solution (31.9 mEq/L) or a normal serum (25.6 mEq/L) at the indicated percentages. Measured bicarbonate levels in the admixtures were plotted together with the predicted bicarbonate values in the admixtures calculated from the sum of the individual component contributions.

of Kreb cycle intermediates such as citrate and isocitrate, acetaminophen toxicity or 5-oxoprolinase deficiency and propylene glycol have been reported [5–9]. We excluded the above-mentioned possible causes of HAG metabolic acidosis, with appropriate blood and urine tests.

Lowering of the serum bicarbonate level without any alteration in other elements of the chemistry profile will *de facto* result in an apparent widening of the normal anion gap by pure mathematical means. The resulting diagnosis of an HAG metabolic acidosis is contingent upon valid bicarbonate measurement. Factitious reduction in laboratory measures of serum bicarbonate levels due to interfering substances or serum-specific factors has, to our knowledge, never been reported.

The inhibition of serum bicarbonate in this case does not appear to be due to an excess of a soluble inhibitor or interfering substance. The suppression of bicarbonate quantitation was neither reversed by dilution nor transferred to a mixture of patient serum with a second source of bicarbonate. The patient's bicarbonate appears to be at least partially inaccessible to the enzyme reactions in the bicarbonate assay, yet accountable in the indirect calculation using the Henderson–Hasselbach equation. At this time, the nature of the inhibition remains unknown.

Clinicians depend upon the veracity of the assay for serum bicarbonate to make accurate assessment of acid–base disturbances. As demonstrated here, factitious reduction in serumHCO_3^- levels coupled with an HAG can occur and should be a consideration in an otherwise healthy-appearing individual in whom organic acids are not present in excess.

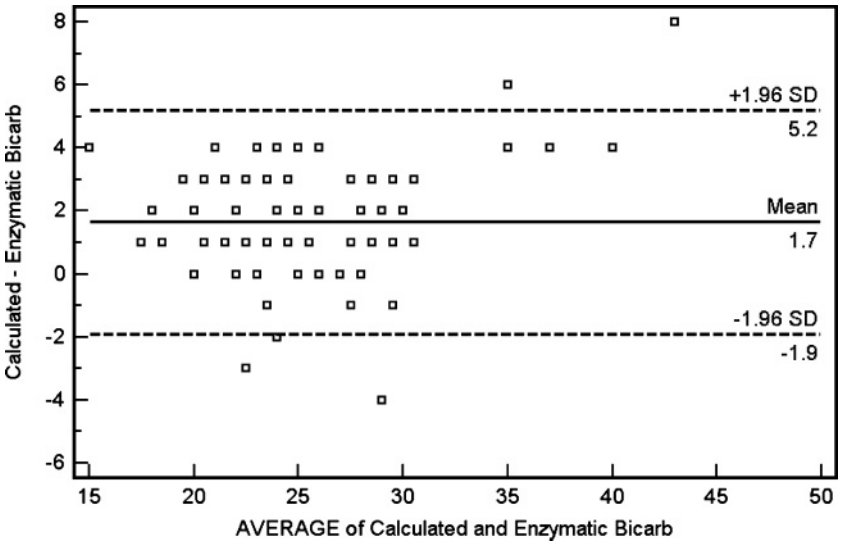

Fig. 2. Bland–Altman plot of the difference between calculated bicarbonate and enzymatic measurement of serum bicarbonate. Data represent a series of 88 paired patient data sets of calculated bicarbonate, derived from the Henderson–Hasselbalch equation applied to arterial blood gas parameters and a matched serum bicarbonate measurement. Units are mEq/L. Solid line represents the mean difference or bias of 1.7 mEq/L. Several data points are superimposed.

Conflict of interest statement. None declared.

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