Serum β-Hydroxybutyrate Measurement in Patients With Uncontrolled Diabetes Mellitus

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Objective: To assess whether routinely measuring serum β-hydroxybutyrate (β-OHB) concentration might help judge the severity of or assist in treating patients with suspected ketoacidosis.

Methods: Serum β-OHB level was estimated by a standard enzymatic method in 64 episodes in adults admitted to a municipal hospital. Of the 85 specimens analyzed, 60 were taken before treatment from a nearly consecutive group of diabetic patients with ketosis, 21 were follow-up specimens, and 4 were from nondiabetic patients with ketosis.

Results: In the 85 specimens, the correlation between serum carbon dioxide and β-OHB levels was −0.69, and that between anion gap and β-OHB level was 0.75. For just the initial specimens, the respective correlation coefficients were −0.60 and 0.52.

Conclusions: The correlations between serum β-OHB and carbon dioxide levels and the anion gap were close, but not sufficiently so for the β-OHB measurements to be routinely useful alone to assess the severity of the ketoacidosis. Full laboratory assessment of the severity and characteristics of ketoacidosis also requires knowledge of serum carbon dioxide level, anion gap, often blood pH, and ideally serum acetoacetate and lactate concentrations as well as serum β-OHB concentration.

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The inverse relationship between serum CO2 and β-OHB is depicted in Figure 1, with different symbols identifying initial, follow-up, nonacidotic ketosis, and complicated DKA, and the 4 episodes in the patients without diabetes. The correlation coefficient (r) was −0.69 for all 85 specimens (64 initial and 21 follow-up) and was −0.60 for just the initial data in the uncomplicated plus complicated episodes. Several patients had initial CO2 values either well above or below the general trend. Three of the 5 whose serum CO2 level was high in relation to β-OHB were believed to have concurrent metabolic alkalosis owing to severe vomiting, and they also had hypochloremia. In contrast, the 3 instances in which CO2 level was particularly low in relation to β-OHB were in posttreatment specimens with hyperchloremia. Indeed, most of the follow-up CO2 values fell below the regression line (Figure 1) because serum CO2 level did not rise as much as β-OHB level declined with treatment, owing to the sodium chloride–induced relative hyperchloremia. The 9 patients with nonacidotic ketosis had mild
PATIENTS AND METHODS

The concentration of β-OHB was measured in leftover aliquots of serum retrieved from the hospital clinical laboratory’s cold room, usually within a day of admission, in 60 episodes of uncontrolled diabetes mellitus (in 50 patients) and 4 episodes of ketoacidosis in patients without diabetes. The Table indicates the types of episodes in which specimens were analyzed. We included nearly all of the adult patients with diabetic ketoacidosis (DKA) hospitalized at Jacobi Medical Center, Bronx, NY, from July 1996 to July 1997. They had serum glucose levels greater than 13.9 mmol/L (250 mg/dL); CO₂ concentration less than 20 mmol/L and/or AG (calculated as serum [Na⁺ − (CO₂ + Cl⁻)]) greater than 16 mmol/L; and a strongly positive sodium nitroprusside reaction in serum diluted at least 1:2. In the 43 episodes that were “uncomplicated,” the initial total serum CO₂ concentration averaged 12.2 ± 5.6 mmol/L (mean ± SD); the AG, 24.4 ± 6.5 mmol/L; and serum β-OHB level, 9.1 ± 2.6 mmol/L. Follow-up specimens were obtained in 17 episodes (total of 21 specimens), usually after significant biochemical improvement had occurred. The 8 “complicated” episodes included 1 patient (2 episodes) with chronic renal failure, 3 patients (4 episodes) with recent alcoholic binges, and 2 with shock and probably also lactic acidosis. Nine other patients with uncontrolled diabetes had positive serum sodium nitroprusside reactions but not significant acidosis (referred to as “mainly hyperglycemic”); and 4 patients were not diabetic, 3 with alcoholic ketoacidosis and 1 with starvation ketosis. The diabetic patients were treated with currently standard low-dose insulin and hydration protocols⁶⁻⁸ and the study was approved by our institutional review board.

The concentration of β-OHB in serum was measured with an analyzer (Cobas-bio-centrifugal analyzer, Roche Diagnostics, Somerville, NJ), with the use of an established liquid reagent (Sigma Chemical Co, St Louis, Mo) containing β-OHB dehydrogenase and nicotinamide-adenine dinucleotide, based on a method described by Williamson et al.⁹ The formation of reduced nicotinamide-adenine dinucleotide, monitored by measuring the 340-nm absorption, was directly proportional to the β-OHB concentration in the serum sample. In serum stored at 10°C, β-OHB was found to be stable for at least 2 months. Serum electrolyte concentrations were measured by the hospital routine clinical chemistry laboratory by means of a chemical analyzer (Beckman-ASTRA, Brea, Calif). Statistical calculations were made by standard methods.

Well above or below the general trend. Four of the 6 whose AG seemed disproportionately high in relation to β-OHB level had another abnormality that may have contributed to the increased AG. In those 4 cases, the presence of hypotension or severe ethanol intoxication strongly suggested concomitant lactic acidosis. In contrast, patients had a disproportionately low AG compared with the β-OHB level, but without an obvious explanation. The data were below the regression line in nearly all of the follow-up specimens, possibly reflecting the posttreatment decreases of serum protein and phosphate levels.

Arterial blood pH was measured concurrently with venous serum β-OHB and CO₂ before treatment in only 21 episodes. The correlation coefficient between pH and β-OHB was −0.38, not as close as that between either CO₂ and β-OHB (−0.74) or AG and β-OHB (0.51) in these 21 episodes.

The serum sodium nitroprusside reaction, which detects acetocetate but not β-OHB, was positive in all the elevations of serum β-OHB level (1.05–3.20 mmol/L) and positive serum sodium nitroprusside reactions.

The direct relationship between serum AG and β-OHB level is depicted in Figure 2, with r = 0.75 for all 85 specimens (64 initial and 21 follow-up) but only 0.52 for just the initial data in the uncomplicated plus complicated episodes. Several patients had AG values either

Table: Types of Episodes of Ketoacidosis With Serum β-Hydroxybutyrate Measurements

<table>
<thead>
<tr>
<th>Types of Episodes</th>
<th>Episodes</th>
<th>Specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic patients</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uncomplicated DKA</td>
<td>Only pretreatment specimens</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>Initial and follow-up specimens</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Complicated DKA (with chronic renal failure, alcohol binge, or shock)</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>“Mainly hyperglycemic” without acidosis</td>
<td>9</td>
</tr>
<tr>
<td>Nondiabetic patients</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>64</td>
<td>85</td>
</tr>
</tbody>
</table>

*DKA indicates diabetic ketoacidosis.

Figure 1. The relationship between serum carbon dioxide and β-hydroxybutyrate (β-OHB) in 64 episodes of ketosis. Circles represent uncomplicated diabetic ketoacidosis (solid, 43 initial specimens; open, 21 follow-up specimens); solid triangles, 8 initial specimens in complicated diabetic ketoacidosis; open squares, 9 episodes of nonacidotic diabetic ketosis; and solid squares, 4 episodes of nondiabetic ketoacidosis.
patients initially tested except 2 with alcoholic ketoacidosis who were not diabetic but had serum β-OHB levels of 3.6 and 4.0 mmol/L. In several of the DKA cases, the test was not done initially because the specimens were hemolyzed, but the test result was positive subsequently.

**COMMENT**

The data in Figures 1 and 2 indicate, as expected, that serum total CO₂ level and AG correlate with serum β-OHB levels in patients with DKA (overall $r = -0.69$ and 0.75, respectively). The correlations are not closer because patients with DKA may have other biochemical abnormalities that can also lower serum CO₂ levels and raise the AG. The main one is the presence of acetoacetate, whose ratio to β-OHB in DKA ranges from 0.8 to 0.2, and even as low as 0.1. A few patients with DKA also have coexisting lactic acidosis, usually from a concurrent serious illness such as bacteremia or myocardial infarction. Also, patients who are severely dehydrated may have hyperproteinemia, which can elevate the serum AG. In addition, some diabetic patients have chronic renal failure, as in 2 episodes in this series. Some ketoacidotic patients also have a component of hyperchloremic acidosis even before treatment, and hence a greater than expected decline of serum CO₂ level. On the other hand, some have severe vomiting with concurrent metabolic alkalosis and a less than expected decline of serum CO₂ level (or sometimes even a normal or elevated value). This is more likely to occur in alcohol abusers, as in 3 episodes in this series.

The fairly close correlations between CO₂ and β-OHB levels ($-0.69$) and AG and β-OHB levels ($0.75$) for the entire group were biased by the follow-up specimens, among which $r = -0.80$ for CO₂ and 0.90 for AG. However, for just the initial specimens in the patients with DKA, both uncomplicated and complicated, the correlations were only $-0.60$ for that between CO₂ and β-OHB levels and 0.52 for that between AG and β-OHB levels. Our correlation between CO₂ and β-OHB levels was closer than the $-0.36$ found by Porter et al with a similar method. However, the data in Figures 1 and 2 show too much variability between the CO₂ concentration and the changes of CO₂ and AG to permit reliance on any single one of those measurements to predict the severity of the ketoacidosis.

Several authors have reported their experience with the Ketosite (Miles, Sankyo Co, Tokyo, Japan) or a similar method for rapidly estimating serum β-OHB level by reflectance meter. With diluted serum, that method correlated well with enzymatic measurements. However, even if our β-OHB values had been available very quickly, that would usually not have been of much additional diagnostic help and probably would have had little therapeutic significance in most of the diabetic patients, although the Ketosite method was recently used by Wiggam et al to guide treatment. Currently available routine laboratory measurements (serum glucose level, sodium nitroprusside reaction, total CO₂ level, and AG) usually permit rapid diagnosis of DKA, and the severity of the acidosis is best judged by the AG and blood pH.

Although the AG was misleadingly low in a few of our patients, the usually low serum CO₂ level, strongly positive sodium nitroprusside reactions, and severe hyperglycemia led quickly to the correct diagnosis in most of the diabetic patients. In any case, faced with a diabetic patient with severe hyperglycemia and a metabolic acidosis with increased AG, especially if the patient omitted therapy, the prudent physician will begin treating for possible DKA.

In the past, when it was believed that the amount of insulin needed to treat DKA should parallel the severity of hyperglycemia or hypocarbia or the serum sodium nitroprusside titer, it might have been useful to know the concentration of serum β-OHB (or preferably β-OHB plus acetoacetate), but insulin is no longer given on that basis. We now usually give low doses of insulin, which interrupt increased hepatic ketogenesis, after which metabolism and renal excretion of β-OHB and acetoacetate dissipate the ketoacidosis. In recent years the “fine-tuning” in treating patients with DKA has shifted mainly to other aspects of the disorder. In addition to giving insulin, we now tailor therapy to remedy the dehydration, hyperosmolarity, and acidemia present in each individual, and treat the complicating or precipitating illnesses, especially infection or myocardial infarction. To be sure, there are rare cases of DKA that seem resistant to low doses of insulin, a circumstance usually associated with either uncontrolled infection or neutralizing antibodies.

Simple, accurate, inexpensive, and rapid methods to measure the concentrations of serum β-OHB, acetoacetate, and lactate could help elucidate the nature of some increased AG metabolic acidoses. This includes some patients without diabetes, such as those with alcoholic ketoacidosis, and rare diabetic patients who may have negative or only weakly positive sodium nitroprusside reactions.
despite elevated β-OHB level.\textsuperscript{5,11} Meanwhile, clinical laboratories should consider whether the rapid Ketosite assay for β-OHB should replace the sodium nitroprusside reaction or at least supplement it in patients with negative reactions. We have not used the Ketosite assay, but its reported ease, rapidity, and close correlation with enzymatic measurements\textsuperscript{16-20} may warrant its use to help clarify the causes of some cases of increased AG metabolic acidosis.

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REFERENCES