Capillary Blood Beta-Hydroxybutyrate Measurement by Reagent Strip in Diagnosing Diabetic Ketoacidosis

PUNTIP TANTIWONG, GOBCHAI PUAVILAI, BOONSONG ONGPHIPHADHANAKUL, PONGAMORN BUNNAG, CHARDPRAORN NGARMUKOS

OBJECTIVE: To compare blood beta-hydroxybutyrate (β-OHB) levels measured by reagent strip with serum ketone levels assessed by the nitroprusside reaction in diagnosing diabetic ketoacidosis (DKA).

DESIGN: Prospective study of DKA in 19 Thai diabetic patients from September 2001 until August 2002.

SETTING: A university hospital.

PATIENTS: Nineteen patients with DKA and ten patients with metabolic acidosis from other causes.

INTERVENTIONS: Capillary blood β-OHB was measured by a blood ketone meter (MediSense® Optium™). Concurrently, serum ketone was measured semiquantitatively by nitroprusside reaction (Ketostix®, N-multistix®, and Labstix®).

MAIN OUTCOME MEASURE: Sensitivity, specificity, and ROC curve of both methods in diagnosing DKA.

RESULT: Mean age ± SD of DKA patients was 45.6 ± 16.95 years. Plasma glucose was 675.25 ± 188.15 mg/dL, arterial blood pH 7.19 ± 0.12, anion gap and serum bicarbonate 29.93 ± 4.90 and 8 ± 3.35 mmol/L. Serum ketone was moderately to markedly positive in most cases. Capillary β-OHB ranged from 2.4 to >6 mmol/L. The sensitivity and specificity of serum ketone by nitroprusside reaction in diagnosing DKA were 95% and 100%. The sensitivity and specificity of capillary β-OHB was 90% and 100% respectively. The areas under ROC curves of serum ketone and capillary β-OHB were 0.975 and 0.950 (NS) respectively.

CONCLUSION: Serum ketone and blood β-OHB measurement are equally effective in diagnosing DKA among uncomplicated cases.

ABBREVIATIONS: β-OHB = beta-hydroxybutyrate; DKA = diabetic ketoacidosis; MA = metabolic acidosis; ROC = receiver operating characteristic.

INDEX TERMS: acetoacetate; beta-hydroxybutyrate; diabetic ketoacidosis; serum ketone measurement.

Clin Lab Sci 2005;18(3):139

Puntip Tantiwong MD *is a Fellow in Endocrinology and Metabolism;* ***Gobchai Puavilai MD*** *is Professor of Medicine, Chief, Division of Endocrinology and Metabolism;* ***Boonsong Ongphiphadhanakul MD*** *is Professor of Medicine;* ***Pongamorn Bunnag MD*** *is Assistant Professor of Medicine; and* ***Chardpraorn Ngarmukos MD*** *is Instructor; Mahidol University, Bangkok, Thailand.*

Address for correspondence: *Puntip Tantiwong MD, Department of Medicine, Maharat Nakornratchasima Hospital, Nakornratchasima 30000, Thailand. 66-02-201100, ext 1647, 66-02-2011715 (fax). tpuntip@hotmail.com*

Diabetic ketoacidosis (DKA) is a most serious acute complication of diabetes mellitus (DM). The mortality rate of patients with DKA varied between 4% and 10% in most published series.[1,2,3] The higher mortality rate was found in elderly patients, complicated DKA, and in those with severe associated diseases.[4,5] DKA results from absolute or relative insulin deficiency, in combination with increased levels of counter-regulatory hormones, e.g., glucagon, catecholamines, cortisol, and growth hormone. Lipolysis is enhanced resulting in the production of acetyl Co-A, which is a substrate for hepatic ketogenesis. Elevation of circulating ketone bodies, i.e., beta-hydroxybutyrate (β-OHB) and acetoacetate leads to metabolic acidosis. β-OHB is the main form of the ketone bodies with the concentration two to three times higher than that of acetoacetate.[6]

Because most hospital laboratories do not quantitatively measure acetoacetate or β-OHB levels routinely, clinical diagnosis of ketoacidosis depends on the semiquantitative assessment of ketones in plasma with the nitroprusside dipstick test. This test mainly measures acetoacetate, and is weakly reactive with acetone but does not detect β-OHB. As mentioned earlier, β-OHB is the predominant ketone body in DKA, and the ratio of β-OHB to acetoacetate higher than two to three may

be found when the redox state of hepatocytes is changed, such as during hypoxia or shock. In these situations, semiquantitative tests for ketone bodies by the nitroprusside reaction may underestimate the total amount of ketones in the body. In addition, this assay may give a false positive result caused by drugs possessing a free sulfhydryl group.[7] So the direct β-OHB measurement, although presently not recommended for diagnosing DKA seems to have more clinical benefits than the semiquantitative nitroprusside test for ketones currently used in most clinical settings. The quantitative estimation of the blood β-OHB levels can now be performed easily using reagent strips and a reflectance meter.[8] The aim of this study was to compare the clinical utility of capillary blood β-OHB measurement by reagent strip and reflectance meter with the semiquantitative test for serum ketones by nitroprusside reaction in diagnosing DKA.

MATERIALS AND METHODS

We prospectively studied 29 diabetic patients with metabolic acidosis admitted in the Department of Medicine at Ramathibodi Hospital, from September 2001 to August 2002. Patients were selected based on the feasibility of performing blood β-OHB measurements and semiquantitative tests for serum acetoacetate concurrently. The first group was 19 patients with DKA (DKA group). The inclusion criteria in the DKA group were 1) plasma glucose levels greater than 250 mg/dL, 2) serum bicarbonate levels less than 18 mmol/L, 3) anion gap greater than 12 mmol/L, and 4) ketonemia as assessed by nitroprusside test.[9] A second group included ten diabetic patients with metabolic acidosis due to causes other than DKA (MA group). Patients in this group had plasma glucose levels greater than 250 mg/dL, metabolic acidosis, and serum bicarbonate levels less than 18 mmol/L. Baseline capillary blood β-OHB levels, serum ketone levels measured by a nitroprusside reaction, plasma glucose concentration, serum electrolytes, and arterial blood gases were obtained in every subject.

BIOCHEMICAL MEASUREMENT

Capillary blood β-OHB measurement was performed using a reflectance hand-held meter with an electrochemical blood ketone sensor (MediSense® Optium™, MediSense/Abbott Laboratories, Abingdon, U.K.) whose accuracy in measuring blood β-OHB concentrations compared with an established laboratory enzymatic reference method was previously reported.[8] Five microliters of capillary blood were applied to a ketone strip which was then inserted into the handheld sensor and the β-OHB concentrations were displayed in mmol/L after 30 seconds. Exact guidelines on the interpretation of blood β-OHB levels have not yet been established. The current consensus, however, suggests that levels below 0.6 mmol/L are regarded as normal, levels over 1 mmol/L represent hyperketonemia, and levels in excess of 3 mmol/L indicate ketoacidosis.[10]

Serum ketone was measured semiquantitatively by the nitroprusside reaction using a urine dipstick test (Ketostix®, N-multistix®, and Labstix®, Bayer Diagnostics, Stoke Poges, Slough U.K.). Plasma glucose was measured by a glucose oxidase method.

RESULTS

Twenty episodes of DKA occurred in nineteen patients in the DKA group (Table 1). Eight patients had type 1 diabetes, seven had type 2 diabetes, and no definite classification of diabetes was made in four patients. The classification of diabetes in this study was based on clinical characteristics, i.e., age, body mass index, and dependence on insulin for prevention of DKA. The precipitating causes of DKA in patients with type 1 diabetes were discontinuation of insulin in eight episodes, and malarial infection in one episode. Among seven patients with type 2 diabetes, the precipitating causes were sepsis in seven cases and both stroke and sepsis in three cases. For those patients with an unclassified type of diabetes, two had sepsis, one had alcoholic pancreatitis, and another had a history of excessive intake of soft drinks as precipitating causes.

The MA group consisted of ten diabetic patients, one with type 1 diabetes and nine with type 2 diabetes. The causes of metabolic acidosis among these patients were sepsis in seven cases (one with hypotension), and renal failure in three. Baseline clinical characteristics and metabolic parameters are summarized in Table 2.

Patterns of acid-base disturbances were evaluated in seventeen episodes of DKA. Pure metabolic acidosis was found in ten episodes (58.8%), combined metabolic acidosis and respiratory alkalosis from sepsis in five episodes (29.4%), and combined metabolic acidosis and respiratory acidosis in two episodes (11.8%).

In the DKA group, serum ketone levels measured by the nitroprusside reaction and capillary blood β-OHB levels in twenty episodes of DKA are shown in Figure 1 and Table 1. Serum ketone levels in twelve DKA episodes were largely positive, seven were moderate, and only one was small. Capillary blood β-OHB levels as measured by the blood ketone meter

were more than 3 mmol/L in 18 episodes of DKA. In two episodes, the capillary blood β-OHB levels were 2.9 mmol/L and 2.4 mmol/L. Both patients had moderately positive serum ketone levels. One patient who had a low serum ketone level by the nitroprusside reaction, had a capillary blood β-OHB level of 4.1 mmol/L. During this episode the patient was not in shock nor hypoxic. In one individual whose blood β-OHB level was 2.9 mmol/L, the serum bicarbonate level was 15.2 mmol/L. In another episode where the blood β-OHB level was 2.4 mmol/L, the serum bicarbonate level was 6.7 mmol/L, but the pH was 7.30. In the MA group, serum ketone levels by the nitroprusside reaction and capillary blood β-OHB levels in ten diabetic patients are shown in Figure 2 and Table 1. Serum ketone levels measured by the nitroprusside reaction were negative in eight patients and trace in two patients. Capillary blood β-OHB levels were less than 0.6 mmol/L in nine diabetic patients. Only one patient had a blood β-OHB level of 2 mmol/L.

The sensitivity and specificity of the serum ketone levels measured by the nitroprusside reaction and the capillary blood β-OHB levels in diagnosing DKA were calculated using conventional formulas. The 'gold standard' for diagnosis of

Table 1. Baseline blood ketone levels in DKA and MA groups

DKA group number	Plasma glucose (mg/dL)	Serum HCO_3 (mmol/L)	Anion gap (mmol/L)	Serum ketone (nitroprusside)	Blood β-OHB (mmol/L)
1	652	6.0	30	moderate	6.0
2	733	5.0	31	moderate	4.6
3	697	5.7	29	moderate	5.6
4	618	14.0	21	large	4.3
5	1036	8.8	27	large	>6.0
6	476	5.2	33	large	4.2
7	613	9.3	24	small	4.1
8	436	7.3	32	large	>6.0
9	336	3.4	27	large	>6.0
10	503	12.5	29	large	4.8
11	708	10.0	33	large	6.0
12	815	15.2	26	moderate	2.9
13	589	10.2	27	moderate	5.8
14	550	4.6	28	large	5.0
15	784	6.5	29	large	5.6
16	926	11.7	29	large	5.6
17	526	7.6	31	moderate	6.0
18	932	5.0	39	large	5.6
19	618	5.3	40	large	>6.0
20	957	6.7	24	moderate	2.4
MA group number					
1	575	15.3	25	negative	0
2	1097	18.0	14	negative	0
3	833	13.6	21	negative	0
4	346	9.2	25	trace	0
5	416	12.7	28	negative	0
6	914	13.7	22	trace	0.3

DKA used in this study was the diagnostic criteria for DKA as recommended by American Diabetes Association (ADA).[9] The sensitivity and specificity in diagnosing DKA of serum ketone by nitroprusside reaction, when these levels were moderately to largely positive, were 95% and 100% respectively. The sensitivity and specificity of capillary blood β-OHB when these levels were more than 3 mmol/L were 90% and 100% respectively.

The areas under the receiver operating characteristic (ROC) curves of the tests were 0.975 and 0.950 respectively (Figure 3), which was not statistically different.

STATISTICAL ANALYSIS

Data are presented as mean ± SD in Table 2. The acid-base disturbance was evaluated using the Henderson-Hasselbach equation. Comparisons between groups were analyzed by Fisher's exact test for categorical variables and Mann–Whitney test for continuous variables. Statistical significance was set as $p < 0.05$. Sensitivity and specificity of the semiquantitative plasma ketone test and the capillary blood β-OHB measurement in diagnosing DKA were calculated and compared by assessing the area under the receiver operating characteristic (ROC) curves. All statistical analyses were performed using STATA version 7.0 (Stata Corporation, College Station TX, U.S.).

DISCUSSION

This study compares two methods of blood ketone measurements for diagnosing DKA: the capillary blood β-OHB level by a blood ketone meter and the serum ketone level by the nitroprusside reaction which detects acetoacetate level semiquantitatively, but not β-OHB. We have shown that both methods are equally effective among uncomplicated DKA patients. At the present, the diagnostic criteria for DKA from the ADA clinical practice recommendations include the wide anion gap metabolic acidosis in hyperglycemic patients with positive reaction for serum ketones by the nitroprusside reaction.[9] This criterion seems to be highly sensitive and more false-positives are anticipated because there are many other conditions that may cause some increased ketone production although not high enough to be responsible for acidosis, i.e., fasting, prolonged exercise, and pregnancy. Ideally, the best method to measure the serum ketone levels for diagnosis of DKA should be the total serum ketone measurement. However, the current method for such measurement is generally not available for clinical use, especially

Table 2. Baseline clinical characteristics and metabolic parameters of DKA and MA groups

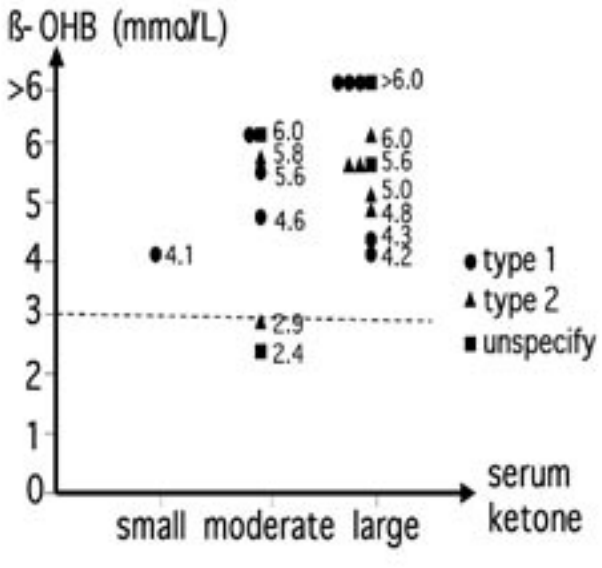
Parameters	DKA group (n = 20) Mean ± SD	Range	MA group (n = 10) Mean ± SD	Range	P value
Sex	11 male:9 female		6 male:4 female		
Age (year)	45.55 ± 16.95	17 - 83	53.1 ± 19.56	22 - 75	NS*
Plasma glucose (mg/dL)	672 ± 188	336 -1036	627 ± 274	346 - 1097	NS
Arterial pH	7.2 ± 0.1	7.0 -7.4	7.2 ± 0.2	6.9 - 7.3	NS
Serum HCO_3 (mmol/L)	8.0 ± 3.4	3.4 - 15.2	13.2 ± 3.9	4.8 - 18	0.0042
Anion gap (mmol/L)	29.93 ± 4.90	21 - 40	22.52 ± 5.59	14 - 33.2	0.0028
BUN (mg/dL)	35.75 ± 26.21	11 - 102	65 ± 49.67	11 - 187	NS
Creatinine (mg/dL)	1.96 ± 0.84	0.9 - 4.6	3.32 ± 1.92	1.2 - 6.3	NS
Serum Na (mmol/l)	132.45 ± 9.02	122 - 150	134.6 ± 11.7	116 - 150	NS
Serum K (mmol/L)	5.17 ± 0.78	4.26 - 7.16	4.69 ± 1.31	2.49 - 6.25	NS
Serum Cl (mmol/L)	94.5 ± 9.13	77 - 177	98.9 ± 11.12	83 - 117	NS
Serum Ca (mg/dL)	9.07 ± 1.60	3.6 - 10.7	8.47 ± 0.74	7.8 - 9.9	NS
Serum P (mg/dL)	4.95 ± 2.38	0.7 - 9.1	3.26 ± 1.79	0.7 - 7.2	NS
Effective serum osmolarity (mOsm/kg)	302.39 ± 21.57	272.4 - 345	304.04 ± 22.06	278.9 - 342.8	NS

*NS = not significant

at the bed side. The introduction of bed side measurement of capillary blood β-OHB, the major ketone body in DKA, by a handheld meter theoretically seems to be a significant improvement in the diagnosis of DKA, especially in conditions associated with an altered redox state and subsequent increased ratios of β-OHB to acetoacetate such as shock and hypoxia.

Umpierrez and coworkers compared levels of β-OHB determined by reagent card using a reflectance meter (Ketosite test, GDS Diagnostics, Elkhart IN) and the standard automated enzymatic technique, with acetoacetate levels and parameters of acid-base status during therapy in 15 DKA patients.[11] They found that the β-OHB levels on admission correlated better than acetoacetate levels with change in acid-base status. At the time when ketoacidosis was resolved by acid-base parameters, 8 of 15 patients had a positive nitroprusside test while all patients had β-OHB levels <0.5 mmol/L. They suggested that the measurement of β-OHB levels was useful in establishing the diagnosis of DKA and in the management of selected patients, such as in those with prolonged metabolic acidosis, combined diabetic and lactic acidosis, and other mixed acid-base disorders. After the initial diagnosis of DKA, the use of the nitroprusside test may be misleading and should be avoided because the fall in acetoacetate level lags behind the resolution of acidosis.

Figure 1. Serum ketone by nitroprusside reaction vs capillary blood β-OHB levels in DKA group



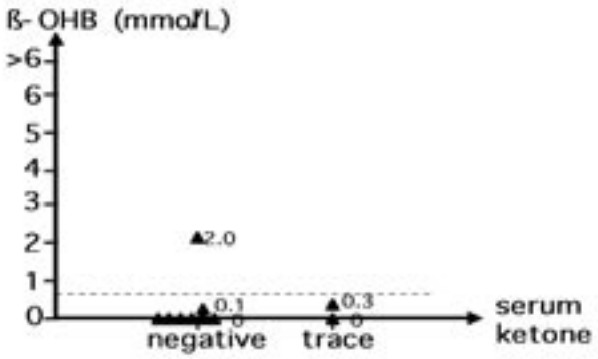
β-OHB levels >3 mmol/L indicate ketoacidosis
β-OHB = beta-hydroxybutyrate, DKA = diabetic ketoacidosis

Porter and coworkers compared the β-OHB levels by the liquid enzyme reagent method (Sigma, St Louis MO) and the qualitative determination of acetoacetate levels (Acetest, Bayer, Elkhart IN) in 45 uncomplicated DKA patients on admission.[12] They found that, at baseline, the higher β-OHB concentrations did not correlate with lower blood pH and serum total CO_2. The acetoacetate and β-OHB levels closely paralleled each other in most patients. It was concluded that β-OHB and acetoacetate measurements were equally effective for the diagnosis of DKA when moderately to largely positive levels of acetoacetate by Acetest were found.

A disparity between the β-OHB and acetoacetate levels without clear explanation was found in three DKA patients. One case with type 1 diabetes (patient number 7) had a small serum acetoacetate level while the β-OHB level was 4.1 mmol/L which is definitely high for diagnosing ketoacidosis. This patient was not in shock nor hypoxic. The other two patients (patients numbers 12 and 20) had moderately positive serum acetoacetate levels while β-OHB levels were less than 3 mmol/L indicating the presence of hyperketonemia, not DKA.

This study showed no superiority of capillary blood β-OHB measurement in diagnosing DKA compared to a semiquantitative test for serum acetoacetate currently used in clinical practice. Both methods of serum ketone measurements are equally correct in diagnosing DKA. However, our findings can be applied only to uncomplicated DKA patients. The patients enrolled in this study were not complicated by

Figure 2. Serum ketone by nitroprusside reaction vs capillary blood β-OHB levels in MA group



Normal level of β-OHB is <0.6 mmol/L
β-OHB = beta-hydroxybutyrate, DKA = diabetic ketoacidosis

shock or hypoxia. A further study that enrolled more DKA patients especially those who had shock or hypoxia to determine the difference in clinical utility of β-OHB and acetoacetate levels measurement for diagnosis of DKA in complicated patients is highly desirable.

It is concluded that the diagnosis of DKA by using the serum ketone measurement by the nitroprusside reaction or by using the capillary blood β-OHB measurement by a blood ketone meter is equally effective among uncomplicated DKA patients.

Because the number of patients who enrolled in this study was limited, a further study including more participants, particularly DKA patients complicated with hypoxia and shock, is warranted.

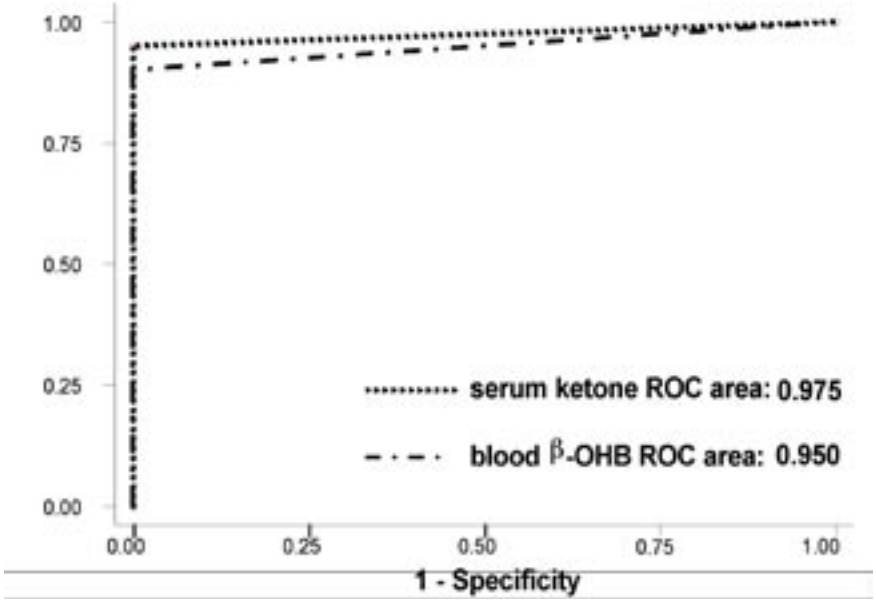
This study was approved by the Ethical Clearance Committee on Human Rights Related to Researches Involving Human Subjects, Faculty of Medicine, Ramathibodi Hospital, Mahidol University. (No. 541/2001 (I))

ACKNOWLEDGEMENTS

The blood ketone meters and the β-OHB strips were provided by Abbott Laboratories, Thailand. We are indebted to the medical residents of Ramathibodi Hospital during 2001 – 2002.

These data were presented at the 14th Annual Meeting of The Endocrine Society of Thailand, on November 7, 2002 at Siam City Hotel, Bangkok, Thailand.

Figure 3. Receiver operating characteristic curves of serum ketone as measured by nitroprusside reaction and capillary blood β-OHB measurement in diagnosing DKA



β-OHB = beta-hydroxybutyrate, DKA = diabetic ketoacidosis

REFERENCES

1. Maclsaac RJ, Lee LY, McNeil KJ, and others. Influence of age on the presentation and outcome of acidotic and hyperosmolar diabetic emergencies. Intern Med J 2002;32:379-85.
2. Bagg W, Sathu A, Streat S, Braatvedt GD. Diabetic ketoacidosis in adults at Auckland Hospital. Aust NZ J Med 1998;28:604-8.
3. Zouvanis M, Pieterse AC, Seftel HC, Joffe BI. Clinical characteristics and outcome of hyperglycemic emergencies in Johannesburg Africans. Diabet Med 1997;14:603-5.
4. Delaney MF, Zisman A, Kettyle WM. Diabetic ketoacidosis and hyperglycemic hyperosmolar nonketotic syndrome. Endocrinol Clin North Am 2000;29:683-705.
5. Lebovitz HE. Diabetic ketoacidosis. Lancet 1995;345:767-72.
6. Stephens JM, Sulway MJ, Watkins PJ. Relationship of blood acetoacetate and 3-hydroxybutyrate in diabetes. Diabetes 1971;20:485-9.
7. Csako G. Consequences and recognition of false-positive reactions for ketones. Clin Chem 1990;36:1388-9.
8. Byrne HA, Tieszen KL, Hollis S, and others. Evaluation of an electrochemical sensor for measuring blood ketones. Diabetes care 2000;23:500-3.
9. American Diabetes Association. Hyperglycemic crisis in patients with diabetes mellitus. Diabetes Care 2001;24 supp 1:83-90.
10. Wallace TM, Meston NM, Gardner SG, Matthews DR. The hospital and home use of a 30 second handheld blood ketone meter: guidelines for clinical practice. Diabet Med 2001;18:640-5.
11. Umpierrez GE, Watts NB, Phillips LS. Clinical utility of beta-hydroxybutyrate determined by reflectance meter in the management of diabetic ketoacidosis. Diabetes Care 1995;18:137-8.
12. Porter WH, Yao HH, Karounos DG. Laboratory and clinical evaluation of assays for beta-hydroxybutyrate. Am J Clin Pathol 1997;107:353-8.