RESEARCH PAPER

Capillary *versus* Serum β-hydroxybutyrate in Pediatric Diabetic Ketoacidosis

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Objective: To find the strength of agreement between point-of-care and serum β-hydroxybutyrate. **Methods:** 236 paired samples (capillary β-hydroxybutyrate by a point of care device and serum β-hydroxybutyrate by colorimetric enzymatic estimation) samples were collected from 26 children aged <13 years admitted with diabetic ketoacidosis. Inborn errors of metabolism and septic shock were excluded. **Results:** Capillary β-hydroxybutyrate showed excellent agreement with serum â-hydroxybutyrate with mean (SD) bias of 0.027 (0.78); 95% limit of agreement -1.51, 1.56 and intraclass correlation 96.1% (*95%CI* 95%–97%, *P*<0.001). An increase in the bias noted for value above 5 mmol/L (*P*<0.001) (serum measurements were higher than capillary point-of-care measure-ments). Capillary â-hydroxybutyrate correlated significantly with blood pH, anion gap,bicarbonate and carbon dioxide levels on blood gas analysis (*P*<0.05). **Conclusions:** Capillary β-hydroxybutyrate estimation is a valid method for monitoring of ketonemia in pediatric diabetic ketoacidosis.

Keywords: *Diagnosis, Ketonemia, Point-of-care, Type-I diabetes mellitus*.

Trial registration: Clinical Trial Registry of India (CTRI/2017/05/008690).

Diabetic ketoacidosis (DKA) is characterized by the triad of hyperglycemia, acidosis, and ketosis. Among blood ketones, beta-hydroxybutyrate (BOHB) predominates in DKA (acetoacetate: BOHB increase up to 1:10 against the normal 1:1), which is the basis of monitoring of blood BOHB in DKA. Current guidelines recommend periodic monitoring of ketone bodies (blood/urine) [1]. Urine ketone estimation, does not provide an accurate estimate of the ketone status as it measures acetoacetate instead of BOHB, and often requires urinary catheterization. Estimation of blood BOHB requires expensive equipment and often fails to provide real-time results. Alternatively, the point-of-care (POC) BOHB meter is a simple handheld device that can work accurately both as capillary glucose and a BOHB sensor [2,3]. The purpose of this study was to describe the strength of correlation and agreement between capillary and serum BOBH and correlation with blood gas parameters.

Methods

The prospective study was undertaken in the Pediatric critical care division in a tertiary-care hospital in Puducherry from July 2015 to July 2017. Approval was obtained from the Institute Ethics Committee of Jawaharlal Nehru Institute of Postgraduate Medical Education and Research.

All children aged <13 years with DKA, as per International Society for Pediatric and Adolescent Diabetes (ISPAD) 2014 definition, were included after written informed consent from parents/legal guardian [1]. Children with suspected/known inborn errors of metabolism (IEM) or having septic shock were excluded. Glycated hemoglobins (HbA1c), venous blood gas, capillary and serum BOHB, blood glucose, serum electrolytes, and renal and liver function tests were obtained at admission and repeated 2-hourly for first first 6 hours, and 4-hourly (or more) till resolution of DKA (except hemogram and HbA1c done only at baseline). Capillary blood glucose was measured every 30-minute till resolution of DKA. 1 mL of serial blood samples for serum BOHB analysis was centrifuged, serum extracted, and stored at -80^{o}C until final analysis. Capillary BOHB measurement was carried out using the point-of-care device (Abbott Optium-H ketone meter, Illinois, USA) after calibration with the calibration stick provided by the manufacturer. Cayman colorimetric enzymatic BOHB estimation kits were used for measurement of serum BOHB [4]. Both methods are based on the quantification

of NADH generated during the enzymatic conversion of BOHB to acetoacetate. A BOHB level of >3 mmol/L was suggestive of ketonemia [1]. Analysis of serum electrolytes, renal and liver function tests were done in the biochemistry laboratory using Olympus AU 680 (Beckman Coulter, California, USA). Blood gas estimation was done using the blood gas analyzer (Cobas b 221 Blood Gas Analyzer, Roche Diagnostics, Switzerland).

The laboratory (reference) method has a coefficient of variation (COV) of 0.98, and point-of-care method has a COV of 0.96 [2,5]. With the power of 95%, and an α-error of 5%, the minimum sample required (i.e., pairs of capillary and serum) was 235, including 10% attrition for hemolysis and laboratory errors. Sample size calculation was done using n-Master version 2.0 (CMC, Vellore, India). Normality of data was checked with Kolmogorov-Smirnov Z test. Cost comparison between POC and serum measurement was made using student *t* test. Intra-class correlation (ICC) with 95% confidence interval (CI) and Bland-Altman plot was used to test the agreement between capillary and serum BOHB. Linear regression was used for evaluating the correlation of capillary and serum BOHB; capillary BOHB with pH, PCO_2, HCO_3, and AG. Two-tailed tests were used and P-value <0.05 considered as statistically significant. SPSS version 20.0 software and Epi Info™ 7 was used for data analysis. The laboratory technician was blinded to capillary BOHB values. The statistician was blinded till preparation of the first draft.

RESULTS

Forty-eight children with DKA were assessed for eligibility (22 excluded IEM=2, missed=14, refused to participate=6), 26 patients were enrolled. A total of 236 pairs of samples were analyzed (39 excluded, hemolysis =30, leaked=9). POC and serum BOHB (mmol/L) value less than one were 55 *vs*. 73, ≥1 <3 was 92 *vs*. 76, ≥3 <5 was 47 *vs*. 47, and ≥5 was 42 *vs*. 40. Twenty one cases of DKA was found to have ketonemia (BOHB ≥3) by POC method with excellent agreement with the reference method (Kappa value 0.752, *P*=<0.001, sensitivity 95.2%). Five cases of DKA were missed by POC method of which one case was diagnosed by the reference method. The baseline characteristics and laboratory parameters are described in ***Table* I**.

The correlation between POC and serum BOHB showed R^2 = 0.863, *P*=<0.001, with a beta-coefficient (slope of the regression line) of 0.929 and intercept of 0.409. The Bland-Altman analysis, as shown in ***Fig.* 1**, showed excellent agreement with a mean (SD) bias of 0.027 (0.78) and 95% limit of agreement is 1.51 to -1.56. An increase in the bias was noted in values above 5 mmol/L (*P*<0.001) (serum higher than POC). The intra-class correlation between POC and serum BOHB was 96.1% (95% CI 95% to 97%, *P*<0.001). POC-BOHB showed a moderate negative correlation with pH (r = -0.563, *P*<0.05) and HCO_3 (r = -0.557, *P*<0.05), weak with pCO_2 (r = -0.378, *P*<0.05) and moderately positive with AG

TABLE I BASELINE CHARACTERISTICS OF CHILDREN WITH DIABETIC KETOACIDOSIS AT ENROLMENT (*N*=26)

Variables	*Patients*	*Variables*	*Patients*
Age, y	8.1 (3.9)	*Serum electrolytes*	
Male: Female, *n (%)*	7 (27): 19 (73)	Sodium, mEq/L	133.3 (6.9)
New onset DKA, *n (%)*	13 (50)	Potassium, mEq/L	4.2 (0.7)
Patients with recurrent DKA (≥2 episodes), *n* (%)	12 (46)	Chloride, mEq/L	106 (8)
Male: Female ratio among recurrent DKA, *n* (%)	2 (17): 10 (83)	*Venous blood gas*	
Weight Z score	-3.0 (2.1)	pH	7.1 (0.1)
Height Z score	-1.3 (1.7)	pCO_2, mm of Hg	22 (6)
Body mass index, kg/m^2	13.05 (1.75)	Bicarbonate, mEq/L	6.7 (3.2)
PRISM III score, median (IQR)	12 (11-12)	Mild DKA, *n* (%)	4 (15)
Modified Glasgow coma scale, median (IQR)	15 (14-15)	Moderate DKA, *n* (%)	7 (27)
Time to hospitalization after first symptom, d	3 (2-7)	Severe DKA, *n* (%)	15 (58)
Serum beta-hydroxybutyrate, mmol/L	4.5 (2.4)	Glycated hemoglobin (%)	12.6 (2.1)
Random blood sugar, mg/dL	475 (99)		
Capillary beta-hydroxybutyrate, mmol/L	4.8 (1.7)		

All data are presented as mean (SD) unless otherwise specified. DKA: Diabetic-ketoacidosis,PRISM: Pediatric risk of mortality

WHAT THIS STUDY ADD?

- Point of care capillary beta-hydroxybutyrate estimation device has good correlation with laboratory beta-hydroxybutyrate estimation, and offers a less costly way to monitor ketonemia in pediatric diabetic ketoacidosis.

(r=0.478, *P*<0.05). The mean (SD) total cost involved in POC-BOHB measurement per patient was significantly lower as compared to laboratory method [₹ 1197 (402) *vs.* ₹ 2903 (976); *P*<0.001]. This cost is exclusive of routine investigations, equipment charges, workforce or other miscellaneous costs. The mean (SD) time for resolution of DKA was 23.5 (13.2) hours.

DISCUSSION

In this study, we documented the excellent correlation and agreement between POC and serum BOHB measurement with an increase in the bias of value above five mmol/L. This can be due to insufficient quantity of reagent on the electrochemical strip and non-linearity of the amperometric detector in the device, and the absolute quantity of acetoacetate may inhibit the enzyme in the test strip or cause inhibition of the quinoid NADH redox mediator incorporated into the electrode [3]. Studies are reported that excellent agreement of POC measurement with standard measurement up to the value of 4 mmol/L [6] and 6 mmol/L [3]. A BOHB value above 1.5 mmol/L indicates that "at risk" for DKA and value ≥3 mmol/L is a diagnosis of DKA [1,6]. A BOHB value less than 1 mmol/L is one of the endpoints of DKA treatment. The clinically relevant range of BOHB value is <1-4 mmol/L [7]. Hence, POC device can be used as a reliable bedside method of ketone (BOHB) estimation, provided that values above 5 mmol/L be viewed with caution. In children, obtaining a urine sample is impractical for ketone estimation, POC-BOHB has a unique value and also can be utilized even in a primary healthcare set-up, as no new equipment is required (the glucometer can be recalibrated as a ketone meter with the help of a calibration stick).

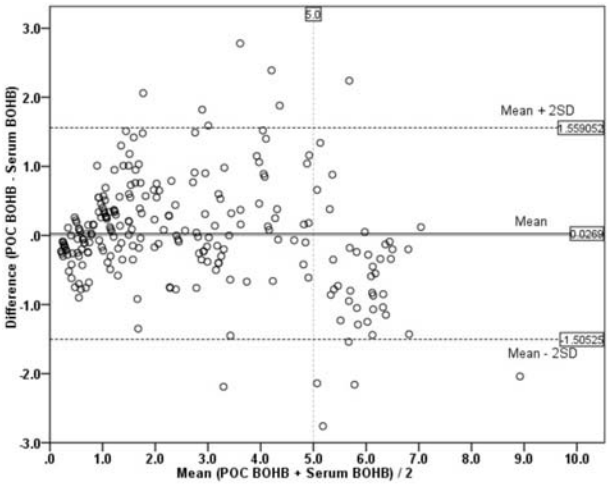

FIG. 1 *Bland-Altman plot between capillary and serum β-hydroxy butyrate (BOHB) levels.*

The correlation of POC BOHB with blood gas parameters has been the subject of evaluation in similar studies [6,8,9] except lower correlation noted with bicarbonate, which is similar to our study results. The findings of this study thus indicate that measurement of serum BOHB can indicate the general trend of disease progression and resolution. POC-BOHB has potential in obviating the need for blood gas. However, if the value is above 5 mmol/L, blood gas analysis is still a necessity. Newer generations of POC-BOHB meters with higher measurement ranges may be able to solve this problem.

The study was limited by fewer samples with BOHB value above 5 mmol/L, which makes it difficult to comment on the accuracy of POC meters at high values. The findings of our study indicate that future recommendation on the management of pediatric DKA should include POC-BOHB monitoring as a convenient, cost-effective and safe alternative to conventional blood gas estimation.

We conclude that the point of care capillary beta-hydroxybutyrate estimation is as accurate as laboratory estimation and has a significant correlation with blood sugar and blood gas parameters, thus making it a reliable tool for monitoring of ketonemia in the management of pediatric diabetic ketoacidosis.

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Contributors: PMK, RR, PS: were involved in the management of the patients; KP: collected the data, reviewed the literature and drafted the first manuscript; PS: contributed for protocol development, review of literature and manuscript; SR:

participated in protocol preparations and drafting of the manuscript and supervised the analysis of biochemical samples; RR: conceptualized the study, reviewed the literature and critically reviewed the manuscript. All authors approved the final version of the manuscript; RR: is the guarantor of the paper.

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