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RESEARCH ARTICLE

Serial correlation between saliva and blood beta-hydroxybutyrate levels in children commencing the ketogenic diet for epilepsy

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Abstract

Objectives: Accurate and user-friendly methods to measure beta-hydroxybutyrate (BHB) concentration are needed to guide the optimal use of ketogenic diet therapy (KDT). We aimed to determine the correlation between serum, capillary, and salivary BHB concentration, and to validate an electrochemical salivary BHB point-of-care test (POCT) in children commencing KDT for drug-resistant epilepsy.

Methods: This was a single center, prospective cohort study. Children <18 years with drug-resistant epilepsy electively admitted to Shenzhen Children's Hospital to initiate KDT between January 1, 2020 and June 30, 2021, were included. Over the 7-day admission, we collected paired saliva and capillary blood samples twice a day and serum blood samples on the first and last days of admission from each

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participant. Salivary BHB was measured using liquid chromatography mass spectrometry (LCMS) and the POCT. Primary outcome was the correlation between serum and salivary BHB concentration measured using LCMS. Secondary outcomes were the correlation between both the capillary blood and salivary BHB concentration, measured by LCMS, and the POCT device.

Results: Seventy-one serum and 334 capillary blood paired with salivary samples were collected from 42 children (median age 4.5 years, interquartile range 1 to 8 years, 45% female). Salivary BHB measured using LCMS strongly correlated with serum BHB (Spearman's $\rho = 0.910$) and capillary blood BHB concentrations (Spearman's $\rho = 0.865$). Salivary BHB concentration was 6% and 7% of serum and capillary blood BHB concentration, respectively. The POCT demonstrated excellent test–retest reliability when compared with LCMS (ICC(A,k) = 0.983, 95% confidence interval: 0.980–0.986). Salivary BHB concentration measured by the POCT showed good accuracy in predicting the capillary blood BHB concentration within the therapeutic range of 2–5 mM.

Significance: Salivary BHB concentration strongly correlates with both serum and capillary blood BHB concentration. The POCT accurately measures salivary BHB concentration and provides a simple, user-friendly method to guide the use of KDT for children with drug-resistant epilepsy.

KEYWORDS

beta-hydroxybutyrate, drug-resistant epilepsy, ketogenic diet, ketone, point-of-care test, saliva

1 | INTRODUCTION

Epilepsy affects 70 million people worldwide, with the highest incidence in children and older adults.[1] Anti-seizure medications (ASMs) are the mainstay of treatment for epilepsy; however, one third of patients have drug-resistant epilepsy.[2,3] Despite the availability of newer ASMs in the last two decades, the proportion of people with drug-resistant epilepsy remains unchanged.[4] For these patients, non-drug options such as dietary therapies deserve consideration.

The ketogenic diet is a high-fat, low-carbohydrate diet first championed for epilepsy in the 1920s.[5] The effectiveness of dietary therapies for drug-resistant epilepsy is supported by Level 1 evidence.[6,7] In clinical trials, ~50% of patients using dietary therapy reported ≥50% reduction in seizure frequency and 10%–15% became seizure-free.[6,8] Over recent decades, there has been increasing utilization of ketogenic therapies for other indications such as cancer,[9] traumatic brain injury,[10] headache,[11] obesity,[12] diabetes,[13,14] and athletic performance.[15]

The ketogenic diet induces a state of ketosis through the conversion of fatty acids to ketone bodies.[16] Beta-hydroxybutyrate (BHB) is the predominant ketone body produced during ketosis, followed in a 3:1 ratio by aceto-acetate.[17] A small amount of acetone is also produced but mostly excreted via respiration.

Key points

- Salivary beta-hydroxybutyrate (BHB) concentration strongly correlates with serum and capillary BHB concentrations.
- The MX3 LAB, a novel electrochemical point-of-care test, can accurately measure salivary BHB concentration compared to gold-standard liquid chromatography mass spectrometry.
- Measuring salivary BHB using point-of-care devices may be a convenient method for monitoring ketone levels for ketogenic diet therapy at home.

The evidence for a correlation between level of ketosis and seizure reduction is conflicting. Monitoring of ketone levels is used to guide ketogenic diet therapy to avoid ketoacidosis and as an indicator of adherence.[18] When the ketogenic diet is used for epilepsy, the typical range for blood BHB is 2–5 mM.[18,19] Current home-based ketone testing systems use blood, urine, or breath samples. Home

capillary blood BHB testing devices correlate closely with serum concentration.[20] Although accurate, this method requires a finger prick, which causes discomfort and is expensive. Urine testing of aceto-acetate is non-invasive but correlates poorly with blood BHB.[21–23] In addition, it may be difficult to obtain urine samples from individuals with urinary incontinence and young children. Prolonged ketogenic therapy leads to renal conservation of ketone bodies, leading to further inaccuracy.[24] Newer commercially available breath-testing devices detect acetone concentration; however, the relationship between breath acetone concentration and seizure frequency has not been established.[25] A more accurate and user-friendly method to monitor ketone levels at home is needed.

Saliva testing is more convenient and straightforward than urine, breath, or blood testing, and is non-invasive. Salivary BHB measured using liquid chromatography mass spectrometry (LCMS) was approximately one-tenth the concentration of serum BHB in patients with cirrhosis and healthy controls with low level ketosis (serum BHB <1.0 mM).[26] However, the relationship between salivary and serum BHB concentrations within the therapeutic range required for epilepsy treatment has not been established. Although LCMS is regarded as the "gold standard," it is impractical for home-based monitoring because it is performed in the laboratory with long sample processing times and is expensive.

Recent advances in the development of point-of-care tests utilizing saliva for a wide variety of indications provide instant information to guide clinical decision-making in the home.[27] One such system, the MX3 LAB (MX3 Diagnostics Inc.), is a novel handheld electrochemical analysis device developed for the detection of biomarkers in saliva, sweat, and other biological fluids. The MX3 LAB device has demonstrated utility in measuring salivary osmolarity as a biomarker of hydration in children and older adults.[28,29]

We aimed to establish the biological relationship between serum and salivary BHB concentration in children whose BHB is in the therapeutic range for epilepsy management. The secondary aim was to validate the MX3 LAB device for saliva BHB measurement for potential home-based use.

2 | METHODS

2.1 | Participants

Participants were prospectively recruited from Shenzhen Children's Hospital (SCH), Shenzhen, China, between January 1, 2020 and June 30, 2021. The hospital is a tertiary epilepsy care referral center serving a 17 million population. Standard clinical practice at SCH is a 7-day inpatient admission for children newly commencing ketogenic diet therapy. Children younger than 18 years of age, who were electively admitted for commencement of the ketogenic diet orally or by tube feeding for drug-resistant epilepsy, were eligible for inclusion. Patients were excluded if they were receiving glycopyrrolate or octreotide or botulinum toxin to the salivary gland, had previously undergone salivary gland surgery, or if the ketogenic diet was contraindicated.[18]

This study received approval from the human research ethics committee of SCH. Written informed consent was obtained from each child's parent or legal guardian. This study followed the Standards for the Reporting of Diagnostic Accuracy Studies (STARD) reporting guidelines.[30]

2.2 | Treatment initiation and sample collection

Participants were admitted to SCH for 7 days. Ketogenic diet was initiated by fasting until capillary blood BHB reached 2.0 mM (Table S1). Data collected included participant demographic and clinical characteristics including age, sex, epilepsy diagnosis, current and previous epilepsy treatments, nutrition and diet information, comorbidities, and seizure frequency.

Saliva samples (2 mL) were collected by unstimulated passive drool from the participants twice daily. Participants were asked not to eat or drink for 30 min prior to saliva collection. Saliva samples were visually inspected after collection and excluded from analysis if there appeared to be contaminants such as obvious food particles. Each sample was split into 1 mL aliquots. One aliquot was immediately stored ("Unprocessed") and the other was centrifuged to yield clear saliva ("Processed"). Centrifuge was specifically required for samples prior to undergoing LCMS to improve sensitivity. All saliva samples were stored at −80°C until analysis.

When each saliva sample was collected, a paired capillary blood BHB measurement was taken. A serum blood sample was collected with the saliva and capillary blood samples on the morning of the first and last day of admission.

2.3 | BHB measurements in serum and capillary blood samples

Capillary blood BHB concentration was measured using a commercial point-of-care device (Beijing Yicheng Bioelectronics Technology T-1 blood ketone meter, Beijing, China). Serum BHB concentration was measured using the Cayman BHB Colorimetric Assay Kit (Michigan, USA).

2.4 | BHB measurements in saliva samples using LCMS

Processed saliva samples were thawed at room temperature, and 20 μL of saliva were aliquoted to Eppendorf tubes. Ten microliters of 80 μM internal standard solution in methanol was added to all samples followed by 70 μL of methanol. The samples were mixed on ice for 30 min and centrifuged at room temperature at 21 k x g for 10 min. The supernatant was transferred to vials and 80 μL of 0.1% formic acid was added. Quality control samples were prepared the same way using non-ketogenic saliva and spiking 2 μM, 10 μM, and 50 μM of BHB. Calibrants in the range 1–100 μM were prepared in 0.1% formic acid solution. A standard curve was constructed using the response ratio of analyte/internal standard in the range 1–100 μM; 1/X2 weighting was used.

LCMS analysis was performed using Vanquish HPLC chromatographic system (Thermo Fisher Scientific) coupled with DSQ Endura triple quadrupole mass spectrometer (Thermo Fisher Scientific). Chromatographic separation was performed at 40°C using XBridge BEH C18 XP column (2.5 μm, 2.1 × 100 mm) (Waters Corporation). The mobile phase A consisted of 0.1% formic acid in water and mobile phase B was acetonitrile. Gradient elution started at 2% B at a flow rate of 0.2 mL/min. %B was increased from 2%B to 10%B in 1.5 min, reached 95%B in the next 0.5 min, and was maintained at 95%B for 1 min, followed by a reduction to 2%B in 0.2 min. Column was equilibrated at 2%B for 0.8 min. The injection volume was 5 μL.

For MS detection, electrospray ionization ion source was used in a negative detection mode and operated in selected reaction monitoring mode. The ion source parameters were optimized infusing the mixture of 3-hydroxybutyrate and internal standard sodium DL-3-hydroxybutyrate-$^{13}C_4$ at 100 μM concentration prepared in 0.1% formic acid 50% methanol. Optimal detection conditions were determined as follows: ion spray voltage of −3500 V, source temperature of 300°C, vaporizer temperature of 375°C, sheath gas of 35 psi, and aux gas of 10 psi. The collision energy was 20 V for BHB and internal standard. Transitions monitored was m/z 103.1 → 59.2 for 3-hydroxybutyrate and m/z 107.1 → 61.2 for the internal standard. Data processing was performed using TraceFinder 4.1 application (Thermo Fisher Scientific).

2.5 | BHB measurements in saliva samples using MX3 LAB

Measurement using the MX3 LAB device (pictured in Figure 1) followed the manufacturer's instruction. Each sample was thawed to ambient temperature and briefly vortexed, and then 30 μL of sample was pipetted onto a small plastic tray. A test strip was attached to the device and then dipped into the sample. The device requires <0.1 mL of saliva to perform a reading. A reading of BHB concentration was displayed on the device within 60 s. Each saliva sample was measured in triplicate and the average value used for analysis.

FIGURE 1 The MX3 LAB Pro testing strips and saliva collect tray. (A) The MX3 LAB device. (B) Saliva ketone testing strips, the arrow indicates where the saliva sample is placed. (C) Saliva collection tray. Saliva can be sampled directly from the mouth, or a collection tray can be used.

2.6 | Statistical analysis

Shapiro–Wilk test and visual inspection were used to examine the distribution of the blood and saliva BHB concentrations. Nonparametric Spearman's correlation coefficient was used, as the data were not normally distributed. Breusch-Pagan test with F-statistic that does not

require an assumption of normality was performed to assess heteroscedasticity, that is, whether the variability in the data changes with different BHB concentration levels. Fractional polynomial regression with robust variance estimator was used to fit regression models of serum and capillary blood BHB concentration, respectively, on salivary BHB concentration. Lin's concordance correlation coefficient (CCC) and nonparametric Passing–Bablok regression were used to assess the absolute agreements between salivary BHB concentration and serum and capillary blood BHB concentration, respectively. Similarly, the methods were used to evaluate the absolute agreements between fitted BHB concentration from fractional polynomial regressions and the corresponding serum and capillary blood BHB concentration. The point estimates and 95% confidence intervals (CIs) of intercept a and slope b from Passing–Bablok regressions were reported. Similar methods were used to investigate the test–retest reliabilities of the MX3 LAB device and its capability to predict clinically relevant capillary blood BHB bands (Data S1).

Serum and capillary blood BHB measurements outside the capillary blood point-of-care device assay range (i.e., <0.3 mM or >6 mM) were excluded from all analyses. Statistical significance level was set at $p < .05$. All statistical analyses were performed using *Stata* version 16.1 (StataCorp).

3 | RESULTS

Forty-six children were enrolled; however, four did not provide samples and were excluded. Demographic and clinical characteristics of the remaining 42 ($n = 19$ [45%] female) children included in the analyses are summarized in Table 1. Unintentionally, all children received ketogenic diet therapy orally, as no children requiring tube feeding were admitted during the study recruitment period. After excluding samples that appeared to be contaminated on visual inspection, 71 serum BHB and 334 capillary blood BHB measurements were collected with paired saliva samples. Of these, 10 serum and 20 capillary blood BHB measurements were outside the assay range of the capillary blood point-of-care device and were also excluded.

TABLE 1 Clinical characteristics of participants.

	***N* = 42**
Median age, years (IQR)	4.5 (1–8)
Female sex, *n* (%)	19 (45)
Primary diagnosis	
Epilepsy type, *n* (%)	
Focal	19 (45)
Generalized	13 (31)
Combined focal and generalized	3 (7)
Unclassified	7 (17)
Epilepsy etiology, *n* (%)	
Genetic	18 (43)
Infectious	1 (2)
Unknown	23 (55)
Previous treatment, *n* (%)	
ASM(s)	39 (93)
ASM(s) + VNS	1 (2)
No treatment	2 (5)
Number of ASMs	
0	1 (2)
1	4 (10)
2	11 (26)
3	13 (31)
4	10 (24)
5	3 (7)
Age at epilepsy diagnosis years, median (IQR)	1 (0–3)
Body mass index, kg/m^2, mean (SD)	17 (2.9)
Ketogenic diet therapy ratio, *n* (%)	
4:1	6 (14)
3:1	11 (26)
2:1	24 (57)
MAD	1 (2)

Abbreviations: ASM, antiseizure medication; IQR, interquartile range; MAD, modified Atkins diet; SD, standard deviation; VNS, vagus nerve stimulation.

3.1 | Correlation between LCMS-measured salivary and serum BHB concentration

Salivary BHB from processed saliva measured by LCMS demonstrated a strong monotonic correlation with serum BHB (Spearman's $\rho = 0.910$). A proportional relationship between the serum and salivary BHB with intercept $a = -0.024$ (95% CI: −0.034 to −0.013), slope $b = 0.063$ (95% CI: 0.046–0.075) was observed, that is, salivary BHB measurement was on average 6% of serum BHB (Figure 2A). Increased variability was observed for higher BHB concentrations ($p = .012$).

A fractional polynomial regression without a constant term fitted the relationship between serum and salivary BHB measurements. The model has $R^2 = 0.941$. The predicted serum BHB concentration demonstrated a strong monotonic correlation (Spearman's $\rho = 0.910$) and good agreement (intercept $a = 0.102$, 95% CI: −0.083 to 0.311, and slope $b = 0.940$, 95% CI: 0.856–1.033; Lin's CCC = 0.909,

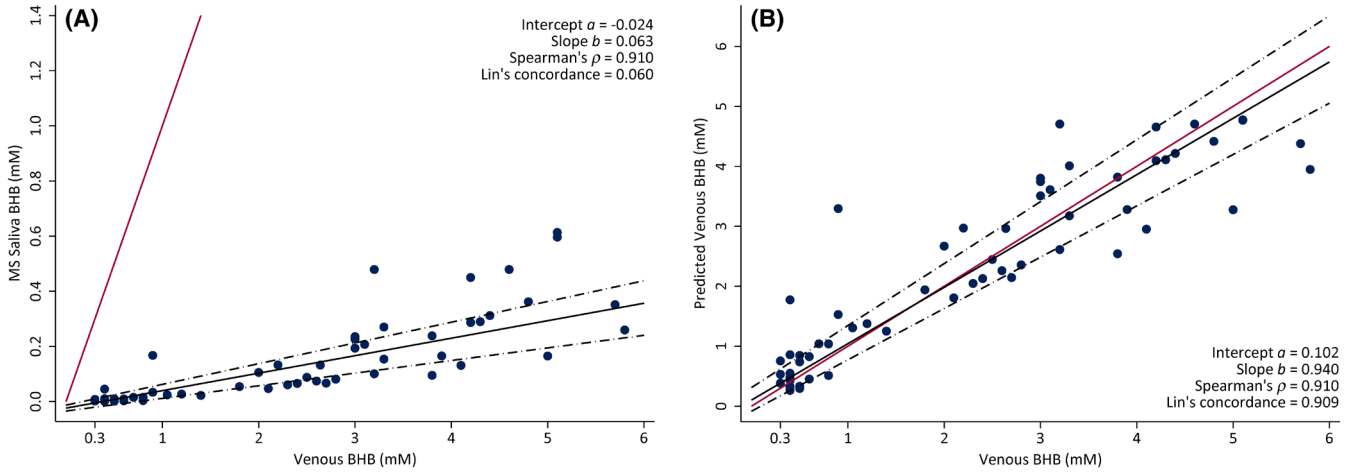

FIGURE 2 (A) Salivary BHB (LCMS) vs serum BHB. (B) Predicted serum BHB using salivary BHB (LCMS) vs serum BHB. (A, B) The solid black line represents the regression line obtained from Passing–Bablok estimation, whereas the dash–dot lines represent the 95% confidence interval. The solid red line represents the diagonal line, indicating perfect agreement. (B) The following fractional polynomial regression without a constant term fitted the relationship between serum and saliva BHB measurements: $E\,(\mathrm{BHB_{serum}}) = 8.38 \times \mathrm{BHB_{saliva}}^{1/2} - 4.77 \times \mathrm{BHB_{saliva}}^{2}$.

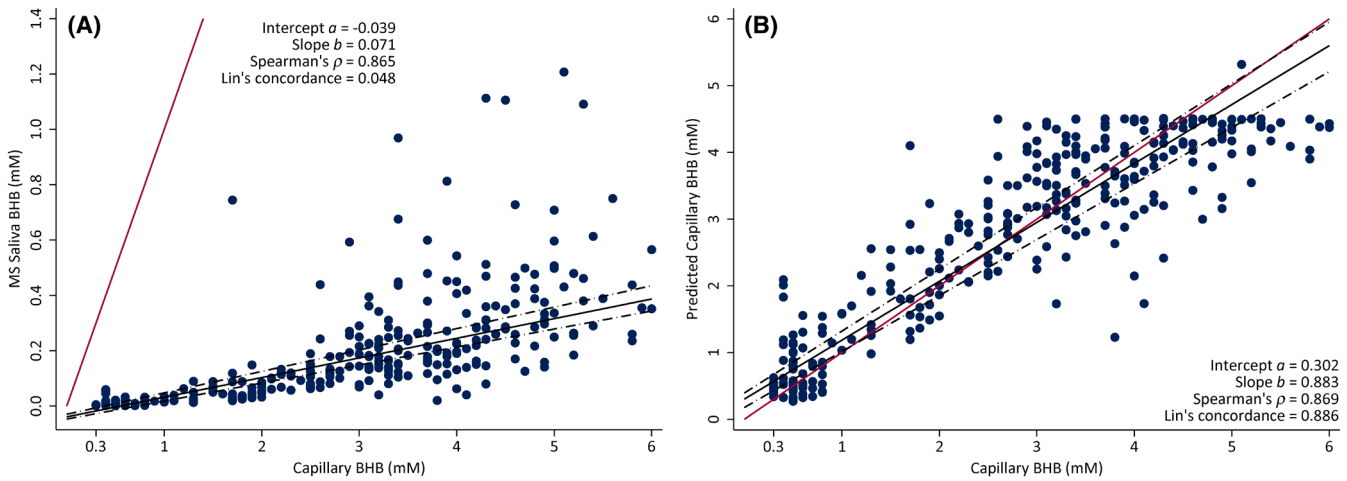

FIGURE 3 (A) Salivary BHB (LCMS) vs capillary BHB. (B) Predicted capillary BHB using salivary BHB (LCMS) vs capillary BHB. (A, B) The solid black line represents the regression line obtained from Passing–Bablok estimation, whereas the dash–dot lines represent the 95% confidence interval. The solid red line represents the diagonal line, indicating perfect agreement. (B) Relationship between capillary blood and salivary BHB measurements was fitted into the following fractional polynomial regression without a constant term: $E\,(\mathrm{BHB_{capillary}}) = 8.62 \times \mathrm{BHB_{saliva}}^{1/2} - 4.59 \times \mathrm{BHB_{saliva}}^{3} + 11.8 \times \mathrm{BHB_{saliva}}^{3} \times \ln(\mathrm{BHB_{saliva}})$.

95% CI: 0.866–0.952) with the observed serum BHB concentration (Figure 2B).

3.2 | Correlation between LCMS-measured salivary and capillary blood BHB concentration

Salivary BHB from processed saliva measured by LCMS also demonstrated a strong monotonic correlation (Spearman's $\rho = 0.865$) but poor absolute agreement with capillary blood BHB concentration (Lin's CCC = 0.048, 95% CI: 0.039–0.057). On average, salivary BHB was estimated to be 7% of capillary BHB concentration (intercept $a = -0.039$, 95% CI: −0.047 to −0.030, and slope $b = 0.071$, 95% CI: 0.065–0.077) (Figure 3A).

Relationship between capillary blood and salivary BHB measurements was fitted into a polynomial regression without a constant term. The model has $R^2 = 0.950$. The predicted capillary BHB concentration demonstrated a strong monotonic correlation (Spearman's $\rho = 0.869$) and moderate agreement (intercept $a = 0.302$, 95% CI: 0.177–0.394, and slope $b = 0.883$, 95% CI: 0.839–0.927; Lin's CCC = 0.886, 95% CI: 0.863–0.909) with observed capillary

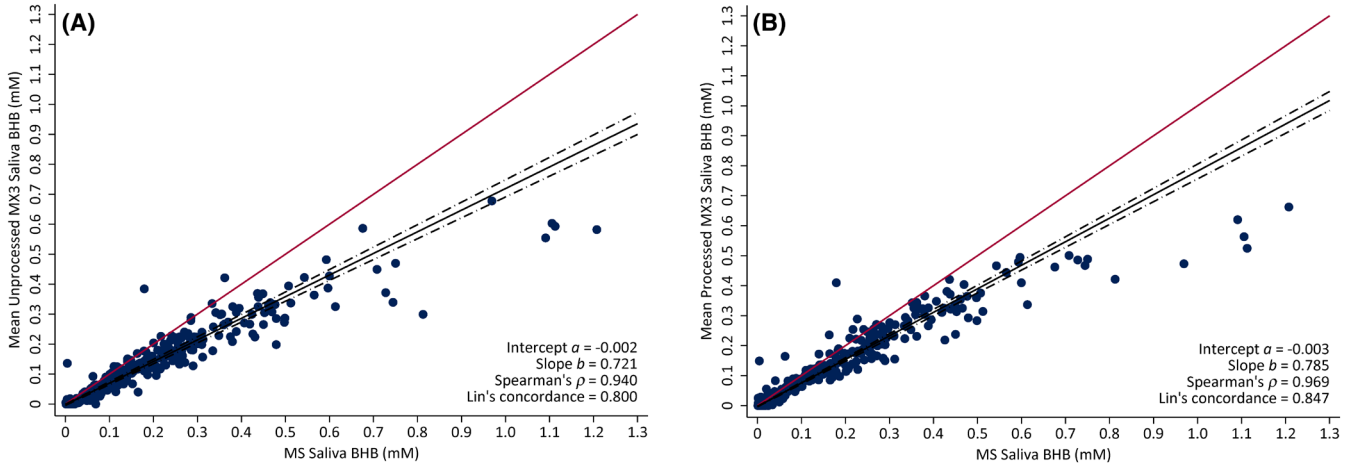

FIGURE 4 Agreement between salivary BHB measurement by LCMS compared to (A) unprocessed and (B) processed saliva samples tested using the MX3 LAB. (A, B) The solid black line represents the regression line obtained from Passing–Bablok estimation, whereas the dash–dot lines represent the 95% confidence interval. The solid red line represents the diagonal line, indicating perfect agreement.

blood BHB concentration. The predicted capillary BHB was estimated to slightly overvalue the actual capillary blood BHB at low concentration and undervalue it at high concentration (Figure 3B).

3.3 | Agreement between MX3 LAB and LCMS-measured salivary BHB concentration

A total of 313 Processed and 285 Unprocessed saliva samples were collected. MX3 LAB device demonstrated excellent test–retest reliability on both processed (ICC(A,k) = 0.983, 95% CI: 0.980–0.986) and unprocessed (ICC(A,k) = 0.976, 95% CI: 0.970–0.980) saliva samples.

The mean BHB concentration measured from unprocessed saliva was estimated to be 72% of the BHB concentration measured using LCMS (intercept $a = -0.002$, 95% CI: −0.005 to −0.0004, and slope $b = 0.721$, 95% CI: 0.696–0.749). The two measurements showed moderate agreement (Lin's CCC = 0.800, 95% CI: 0.772–0.828) (Figure 4A). A similar relationship was observed between the mean processed and LCMS BHB measurements (intercept $a = -0.003$, 95% CI: −0.004 to −0.002, and slope $b = 0.785$, 95% CI: 0.760–0.807; Lin's CCC = 0.847, 95% CI: 0.826–0.869) (Figure 4B).

Fitting the relationship between the raw MX3 LAB voltage signal readings (mV) and LCMS-measured salivary BHB concentration using fractional polynomial regression demonstrated good performance in predicting LCMS-measured salivary BHB concentration in both training and testing sets, using both unprocessed (Figure S1) and processed (Figure S2) samples.

3.4 | Prediction of clinically relevant capillary blood BHB concentration bands

The mean BHB concentration measured from unprocessed saliva was estimated to be 5.5% of capillary BHB concentration (intercept $a = -0.039$, 95% CI: −0.051 to −0.028, and slope $b = 0.055$, 95% CI: 0.050–0.061) (Figure S3), and the mean BHB concentration measured from processed saliva sample was estimated to be 5.8% of capillary BHB concentration (intercept $a = -0.035$, 95% CI: −0.042 to −0.026, and slope $b = 0.058$, 95% CI: 0.052–0.062) (Figure S4).

Capillary blood BHB bands predicted based on the mean MX3 LAB unprocessed salivary BHB in a fractional polynomial regression demonstrated good overall accuracy in both the training set (0.829, 95% CI: 0.780–0.878) and the testing set (0.877, 95% CI: 0.789–0.965). In the testing set, the predicted BHB band exhibits high sensitivity (0.974) but relatively poor specificity (0.684) for the 2–5 mM band. It showed balanced sensitivity (0.867) and specificity (0.881) in predicting the <2 mM band. However, the performance for the >5 mM band was poor, likely due to the low number of samples in this band (Table 2). Similar performance was observed when using the mean MX3 LAB processed saliva BHB (Table S2). The raw MX3 LAB unprocessed and processed signal values showed comparable performances in predicting capillary blood BHB bands (Tables S3 and S4).

4 | DISCUSSION

We determined the relationship between salivary and blood BHB concentrations in children initiating ketogenic

TABLE 2 Prediction of capillary blood BHB bands using MX3 LAB unprocessed salivary BHB.

Observed capillary blood BHB bands	Predicted capillary blood BHB bands							
	Training set				Testing set			
	<2 mM	2–5 mM	>5 mM	Sub-total	<2 mM	2–5 mM	>5 mM	Sub-total
<2 mM	45	16	0	61	13	2	0	15
2–5 mM	8	144	0	152	1	37	0	38
>5 mM	0	15	0	15	0	4	0	4
Sub-total	53	175	0	228	14	43	0	57
Sensitivity	0.738	0.947	0		0.867	0.974	0	
Specificity	0.862	0.592	0.887		0.881	0.684	0.943	
PPV	0.849	0.823	N/A		0.929	0.860	N/A	
NPV	0.823	0.849	0.829		0.860	0.929	0.877	
Accuracy (95% CI)	0.829 (0.780–0.878)				0.877 (0.789–0.965)			

Abbreviations: BHB, beta-hydroxybutyrate; CI, confidence interval; N/A, not applicable; NPV, negative predictive value; PPV, positive predictive value.

diet therapy. We also validated a novel POCT for salivary BHB measurement against the gold standard of LCMS, and determined the accuracy of the salivary BHB POCT in predicting capillary blood BHB concentrations within the established therapeutic range for children treated with the ketogenic diet.

We found that there was a very strong correlation between both serum and capillary blood BHB with salivary BHB concentration measured by LCMS. Salivary BHB concentration was 6% and 7% of serum and capillary blood BHB concentration, respectively. Our larger study adds substantial knowledge to a previous report of tests of saliva in four healthy adults who ingested a ketone supplement. Using a disposable electrochemical strip, their capillary blood BHB concentration was up to 2.0 mM, less than the therapeutic range for epilepsy treatment.[31]

After establishing the biological relationship between salivary and blood BHB concentrations, we tested the accuracy of a novel saliva POCT for BHB. There was excellent agreement between salivary BHB concentration measured by LCMS and the MX3 LAB device. Little difference was observed between processed and unprocessed saliva samples, indicating centrifuging of the saliva samples for LCMS analysis did not result in a loss of BHB. The MX3 LAB device is therefore suitable for direct sampling of whole saliva without any additional preparation.

To assess its potential clinical utility, we determined the accuracy of the salivary BHB concentration measured by the MX3 LAB device in predicting the capillary blood BHB concentration within the therapeutic range. The MX3 LAB demonstrated high sensitivity for predicting capillary blood BHB within the relevant clinical subtherapeutic (<2 mM) and therapeutic (2–5 mM) ranges. This demonstrates the potential clinical utility of the MX3 LAB device for salivary BHB testing.

The international recommendations[18] for the management of children on dietary therapies for epilepsy state that urine ketones should be checked several times per week. Blood BHB concentration correlates better with seizure reduction than urine ketone concentration,[23] with 44% of members of the expert consensus group routinely recommending home capillary blood BHB monitoring.[18,32] Given the accuracy of our salivary POCT, it may now become the optimal test for routine monitoring of ketogenic therapy. As it is non-invasive, this method may also be easy for parents to use by directly sampling saliva from a child's mouth using the testing strip. Future studies are required to establish the acceptability by parents and children, impact on clinical outcomes, and cost implications of salivary BHB testing.

5 | STRENGTHS AND LIMITATIONS

The strengths of our study are the large number of saliva samples analyzed across the clinically relevant range of serum BHB concentrations. The MX3 LAB POCT was compared to quantification using LCMS (gold standard) and against current standard practice of using capillary blood BHB testing. Although only children with epilepsy were included in this study, we would expect salivary BHB testing would be suitable for all patients requiring ketogenic therapy, including adults.

A limitation of this study was that infants could not be recruited, as we could not obtain the 2 mL saliva samples required. Therefore we cannot be certain that the capillary blood and salivary BHB concentration correlate in this age group. In practice, this might be addressed by direct sampling from the mouth. There were a limited number of

saliva samples paired with a blood BHB concentration of >5 mM. Further validation of MX3 LAB device for blood BHB concentrations of >5 mM is required. Finally, the participants were all children initiating ketogenic therapy. It remains unknown whether the correlation between capillary blood and salivary BHB concentration remains stable over time, given the observed renal conservation of ketone bodies with prolonged ketogenic diet use.[24] We acknowledge the conflict of interest of authors M.E. and E.S., who are affiliated with the manufacturer of the MX3 LAB device. To mitigate this issue, their role in the study was technical support only; they were not involved in recruitment of participants, sample collection, or analysis or data analysis.

6 | CONCLUSION

Salivary BHB concentration correlates well with serum and capillary blood BHB concentration. The MX3 LAB device accurately detects salivary BHB concentration compared to LCMS. This novel device provides an accurate, non-invasive method of monitoring BHB concentration to guide the implementation of ketogenic diet therapy for children with drug-resistant epilepsy.

AUTHOR CONTRIBUTIONS

Liao and Kwan have full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Concept and design: Kaul, Duan, Cui, Erlichster, Chen, Anderson, Chan, Liao, and Kwan. Acquisition, analysis, or interpretation of data: Duan, Cui, and Chen. Drafting of the manuscript: Kaul and Chen. Critical revision of the manuscript for important intellectual content: Kaul, Duan, Cui, Erlichster, Chen, Anderson, Chan, Scheffer, Skafidas, and Kwan. Statistical analysis: Chen.

Obtained funding: Liao, Scheffer, Skafidas and Kwan. Administrative, technical, or material support: Duan, Cui, Erlichster, Anderson, and Liao. Supervision: Skafidas, Liao, and Kwan.

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CONFLICT OF INTEREST STATEMENT

MX3 Diagnostics manufactures the MX3 LAB the device used in this study. E.S. is a member of the MX3 Diagnostics Board of Directors. M.E. is an employee of MX3 Diagnostics. E.S. and M.E. have partial ownership of MX3 Diagnostics. E.S. and M.E. are authors of patents related to MX3 products. No other disclosers were reported.

DATA AVAILABILITY STATEMENT

Research data are not shared.

ETHICAL PUBLICATION STATEMENT

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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