Retrospective Audit of Serum Ketone Tests Performed at National Health Laboratory Service Tygerberg Hospital

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ABSTRACT

Introduction: Diabetes mellitus (DM) is characterised by hyperglycaemia as a result of impaired insulin secretion or resistance. A severe complication Type 1 and Type 2 DM is the development of diabetic ketoacidosis (DKA) due to ketone production. Monitoring serum ketone levels aids the diagnosis and management of DKA.

Methods: A retrospective audit was performed to determine the number of serum ketone tests performed at Tygerberg Hospital National Health Laboratory Service (NMLS) over a 6-month period to evaluate the need to introduce β-hydroxybutyrate (β-OHB) testing, possibly as point-of-care test (POCT).

Results: During this period, 253 serum ketone tests were performed, of which 189 were used in this study. Of these, 56% (n=105) were requested for patients with Type 1 and Type 2 diabetes with suspected DKA, of which only 59% (n=62) tested positive. The average turnaround time (TAT) was 5.5 hours with 89% (n=169) exceeding the proposed stability period of 2 hours.

Conclusion: The large proportion of negative results could be due to the instability of ketones at ambient temperature, the significant delay in TAT and the fact that acetoacetate and not β-OHB is measured. A β-OHB POCT device would bridge this gap, but this would first necessitate full method validation.

KEYWORDS: diabetes, ketones, diabetic ketoacidosis, point of care testing

INTRODUCTION

Diabetes mellitus (DM) refers to a group of metabolic disorders characterised by hyperglycaemia as a result of impaired insulin secretion in Type 1 DM, or insulin resistance in Type 2 DM. Type 1 DM is primarily a result of pancreatic islet β-cell destruction and has a tendency to develop ketoacidosis whereas Type 2 DM, in contrast, is characterised by insulin resistance with insulin secretion deficiency.1 The prevalence of DM is increasing worldwide and the management thereof remains challenging.2 The 2017 International Diabetes Federation statistics indicated that DM affects 425 million individuals globally, of which 16 million reside in sub-Saharan Africa (SSA).3 Locally, Erasmus et al described a high prevalence of DM and metabolic syndrome in the Bellville South mixed ancestry population of Cape Town.4 Whereas previously communicable diseases such as human immunodeficiency virus and tuberculosis were the major burden on the healthcare systems in Africa, including South Africa, now non-communicable diseases such as DM and its complications are further depleting already limited resources.4,5
A severe complication especially of Type 1 DM is the development of diabetic ketoacidosis (DKA) in which the breakdown of fatty acids produces ketones. In SSA there are an increasing number of obese, African Type 2 DM patients presenting with ketoacidosis, similar to Type 1 DM. This ketosis-prone Type 2 DM is observed mostly in African populations with a strong male predominance.

Ketones are produced by the metabolism of fatty acids in the liver to provide energy from lipids in times of low carbohydrate availability. The main ketones are acetoacetate (AcAc) and β-hydroxybutyrate (β-OHB), while acetone, another ketone, is formed from spontaneous decarboxylation of AcAc and is present in very low concentrations. Figure 1 displays the biochemical pathway involved in the formation of ketones. Causes of low carbohydrate availability or a decrease in carbohydrate utilisation include DM, starvation, fasting, high fat diets, prolonged vomiting, inborn errors of metabolism, and glycogen storage disease. This leads to accumulation of ketones in blood and development of ketonaemia and ketonuria. When ketone levels exceed the body’s buffering capacity, ketoacidosis occurs.

Dipsticks used for testing serum and urine ketones detect AcAc but not β-OHB, by reacting with nitroprusside. During ketoacidosis, β-OHB levels exceed concentrations of AcAc by as much as 10-fold. Therefore the measurement of AcAc only underestimates the extent of ketonaemia. The limitations associated with urinary ketone testing include delayed urine sampling due to dehydrated DKA patients, lower sensitivity and specificity, and only giving a semi-quantitative measurement of AcAc. Because of these limitations associated with urinary ketone testing it has been suggested that blood ketone measurement may be superior.

The American Diabetes Association (ADA) revised its position on ketone analysis in favour of serum testing and also concluded that capillary measurement is equivalent to venous measurement of β-OHB. β-OHB is measured in serum or capillary blood by using a β-OHB dehydrogenase reaction.

Due to the aforementioned limitations associated with dipsticks ketone testing, serum ketone screening is proposed for the National Health Laboratory Service (NHLS) at Tygerberg Hospital (TBH). Before implementing this change, it is necessary to assess the number of serum ketone tests and if it has been clinically useful. Thus, the purpose of this study was to:

1. perform a retrospective audit of all ketones requests sent to the NHLS TBH over a 6-month period to assess the need for a β-OHB point-of-care test (POCT) device;
2. to assess the turnaround time (TAT) of ketone testing; and
3. to perform a cost analysis of ketone testing.

MATERIALS AND METHODS

The NHLS is the sole provider of diagnostic laboratory services at TBH, which is a tertiary referral hospital with 1384 hospital beds and it also provides service for a large number of regional primary healthcare clinics and hospitals. The study included all serum ketone tests performed at NHLS TBH from 1 June 2017 to 30 November 2017. Samples with no collection time stated on NHLS request form, samples received from referral hospitals, and urine samples were excluded from the study. A retrospective audit was conducted to determine the number of serum ketone tests performed during this period. The TAT of the samples was also examined because of the unstable nature of ketones and the possibility of negatively impacting results.

The NHLS TBH uses Siemens Multistix 10 SG for ketone body determination. These test sticks contain multiple test pads typically used for urine testing, as well as the ketone test pad, containing nitroprusside, which is used for ketone body determination. Testing for ketones is done by placing a drip of serum or urine directly onto the ketone test pad. The concentration of the ketones in the serum or urine sample is proportional to the degree of colour change of the ketone test pad from buff-pink (negative) to purple (positive). The colour change occurs due to the reaction between AcAc and nitroprusside. After waiting for 40 seconds, the colour of the test pad is compared to the colour panel on the Siemens Multistix 10 SG container, and the degree of colour change provides an estimated concentration of present ketones. Data was gathered from the laboratory information system (TrakCare Lab) and compiled into an Excel spreadsheet where it was analysed statistically. TAT was determined by observing the time elapsed between sample collection to registration time, registration to results entered time, and results entered to authorised time.
Ethics approval was obtained from Stellenbosch University Health Research Ethics Committee (HREC-2018-8116).

RESULTS
A total of 255 serum ketone tests were performed during 1 June 2017 – 30 November 2017. The majority of these tests were from TBH inpatients (n=236) with referral hospitals contributing 19 samples. The sample collection time was not stated on 51 of the 236 samples, therefore these were excluded together with the 19 referral samples. The total number of serum ketone tests in this audit amounts to 185. Figure 2 displays the monthly total serum ketone tests performed.

Patient demographics, including sex, age and in-hospital location are represented in Table I. Females accounted for 62% (n=118) and males for 38% (n=71) of the patients. The majority of patients (55%, n=104) were either in emergency centres, high care or intensive care units at the time of sample collection. A large proportion of TBH patients tested for serum ketones included both Type 1 DM (35%, n=66) and Type 2 DM (56%, n=105). Of these patients, 56% (n=105) were in suspected DKA. The remaining 10% (n=18) of patients did not have diabetes (Figure 3).

A total of 107 (57%) of serum samples, including patients in presumed DKA, tested negative for serum ketones. Only 43% (n=82) of all tests had positive readings ranging from “Trace” (5 mg/dL) to “Large” (80–160 mg/dL). Of all the patients with Type 1 DM and Type 2 DM presumed to be in DKA (56%, n=105), only 59% (n=62) tested positive for ketones. The average TAT of samples from collection to registration in the laboratory at TBH was about 5.5 hours. Only 11% (n=20) of the samples reached the laboratory within 2 hours from collection with delivery of the samples contributing the most to the delay. Average TAT from sample collection to reaching the laboratory was 5.5 hours with an additional TAT in the laboratory itself averaging 4 hours. The total time from sample collection to reporting of the test result was thus 9.5 hours. The negative results that exceeded the 2 hour stability accounted for 90% (n=96) of all negative results.

DISCUSSION
We found that more than half (57%) of the tests performed using our current method did not detect serum ketones. This discrepancy could possibly be due to a delay in TAT, which affects the sample stability, and to the current assay in use which only measures AcAc and not β-OHB.

DKA is considered an acute and severe complication of DM with high morbidity and mortality. It is important to identify high risk patients with hyperglycaemia and determine whether they have ketoacidosis. DKA is usually diagnosed in the presence of hyperglycaemia, high anion gap metabolic acidosis, and ketosis. The diagnostic criteria for DKA include a serum bicarbonate level ≤18 mmol/L, pH ≤7.30, the presence of ketonuria or ketonaemia, a high anion gap, and a plasma glucose concentration >16.7 mmol/L. Ketones may be measured either in serum, urine or capillary blood and play a vital role in assessing the severity and also progression or resolution of ketoacidosis, specifically DKA. Paradoxically, the levels of AcAc increase while the β-OHB levels decrease in recovering DKA patients.

The levels of AcAc and β-OHB are determined by the ratio between nicotinamide adenine dinucleotide (NAD⁺) and its reduced form, NADH. During fatty acid oxidation, as in DKA patients, β-OHB production is favoured. However, during the DKA recovery, the ratio is reversed, favouring conversion of β-OHB to AcAc. Therefore, using dipsticks which only detect AcAc may give the impression of a worsening clinical picture. β-OHB is therefore the preferred method for DKA diagnosis and monitoring of successful treatment.

An important factor to consider when determining ketone levels is sample stability. A study performed by Yamanishi et al. in 1993 compared AcAc and β-OHB stability and concluded that β-OHB was stable at room temperature and on ice for up to six hours after venesection. AcAc concentration, on the other hand, decreased significantly after only one hour post-venesection. Another study by Peng et al. demonstrated that AcAc concentration at ambient temperature falls by more than 50% within 2 hours.
Currently there are no guidelines within the NHLS concerning the stability of ketones at ambient temperature. More so, literature review demonstrates that very little has been published on the stability of ketones at room temperature. There is however general consensus that ketones, especially AcAc, are unstable analytes.\textsuperscript{15–17} Many laboratories have standard operating procedures for ketone body determination and require specimens to be delivered to the laboratory within 30 minutes on ice, to prevent degradation. The large proportion of negative serum ketone tests reported by NHLS TBH could be attributed to poor TAT, specifically the pre-analytical component thereof. Addressing TAT, specifically prompt delivery of samples to the laboratory, in an already work overloaded environment, is a daunting task. A limitation may be that we performed AcAc testing on serum samples, whereas the manufacturer’s package insert only specifies the use of urine samples.

Blood ketones can be measured in serum, plasma or whole blood samples using either laboratory analysers or finger-prick capillary blood POCT devices. POCT is defined as medical diagnostic testing at or near the time and place of patient care and can be performed at the bedside. This is accomplished using portable and handheld instruments and test kits.\textsuperscript{18} Measurement of ketones using POCT has numerous advantages including rapid quantitative results obtained in real-time allowing instant changes in management. There are however some pitfalls to using POCT, including untrained users who may misinterpret the results, lack of calibration of devices and inadequate quality control programs to ensure reliable results. The POCT method used to determine ketone levels has poor precision above 3 mmol/L and ketone levels above this threshold may falsely underestimate ketonemia.\textsuperscript{4} It is therefore suggested that patients in DKA with β-OHβ >3 mmol/L, need laboratory validated testing for diagnostic and monitoring purposes.

There have been emerging trends in using POCT devices in hospitals and primary healthcare sites to address laboratory-testing associated difficulties, including delayed TAT and also pre-analytical interferences. Concomitantly these shortcomings may lead to delayed decision-making by the attending healthcare provider. Although the availability of POCT does come with its own considerations, e.g. purchase price of equipment, training of staff, maintenance, quality control, it remains an attractive means of testing and investigating patients, especially diabetics.\textsuperscript{19,20} Numerous biomedical manufacturers have developed POCT devices for β-OHβ testing with increasing popularity worldwide. Further, the development of biomedical microdevices with smartphone interaction is gaining attractiveness in DKA diagnosis and management.\textsuperscript{21}

TBH, a tertiary academic training hospital, attends to and treats a large portion of DM patients with DKA. Having the most accurate and relevant diagnostic and management tools available in such a centre is critical. Placing β-OHβ POCT devices in TBH emergency centres, high care and intensive care units, where most ketone determination is performed, could contribute to better patient management. The placement of these devices in the aforementioned units would eliminate TAT as a contributing factor to false negative results, delayed reporting and clinical decision making. The major contributing factor to the delay in TAT in this study was identified as being the delivery of the specimen to the laboratory for testing and not the laboratory itself. More importantly, clinicians at TBH would receive more reliable results when testing for β-OHβ compared to the current validated assays for ketones, which estimate AcAc concentrations.

Currently the cost to test serum or urine ketones at TBH NHLS, performed on a dipstick, amounts to ZAR12.81. Although the dipstick method is a cost-effective option, it adds little value to DKA diagnosis and management. When considering the inaccuracy of this test, specifically when managing DKA patients, the costs amount to far more than just the test itself. Prolonged hospital stay, additional medication and investigations all rapidly escalate the cost implications of an unreliable test.

**CONCLUSION**

The implementation of POCT devices for β-OHβ determination would undoubtedly contribute to improved clinical management of diabetic patients at TBH. Introducing POCT would however first require method validation by the NHLS. Following the method validation process, training programmes should be offered to clinical staff at the relevant units to aid users in interpretation of results. Importantly, ongoing maintenance and quality control of the POCT devices would be necessary to ensure reliable and accurate results.

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**Conflict of Interest**

All authors declare no conflict of interest.

**Disclaimer**

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