RANDOX

THE ROLE OF D-3-HYDROXYBUTYRATE (RANBUT) IN DIABETIC KETOACIDOSIS

OVERCOMING THE CURRENT CHALLENGES IN KETONE TESTING

The Role of D-3-Hydroxybutyrate (Ranbut) in Diabetic Ketoacidosis

1. BACKGROUND

Diabetes Ketone Levels Circulating in the Blood

Type 2 diabetes mellitus (T2DM) has reached epidemic levels, now attaining the status of global pandemic, spreading from developed countries to developing countries. The burden on healthcare systems and epidemiological trends indicate that the prevalence will continue to increase dramatically in the coming years. According to the World Health Organization (WHO), diabetes is estimated to be the seventh leading cause of death globally with 1.6 million deaths attributed to diabetes in 2016 [1].

DIABETIC KETOACIDOSIS (DKA)

Ketosis is a metabolic process that occurs when the body switches from glucose to predominantly fat metabolism for energy production, this happens when carbohydrate availability reaches low levels. The metabolism of fatty acids in the liver results in the production of chemical by products known as ketone bodies or ketones [2]. Ketosis occurs when the body produces more ketones than the liver can process.

DKA is a serious complication of Type I Diabetes Mellitus (T1DM), however can also affect individuals with T2DM. The condition is linked to insulin deficiency and occurs when glucose levels are consistently high and insulin levels are severely low. Due to this imbalance glucose builds up in the blood and the body responds by metabolising fat rather than glucose. DKA is usually one of the first indicators of T1DM.

Ketosis is not normally dangerous and is typical of ketogenic diets which are low in carbohydrates. Ketones however are poisonous when present in high levels leading to ketoacidosis, DKA for example if left untreated can cause damage to vital organs and in some instances may lead to a coma or death [3]. DKA is commonly triggered by an illness, infection or missing insulin treatments [4].

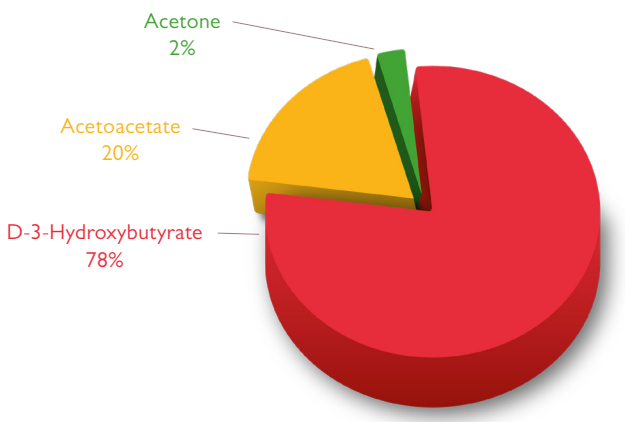
2. CLINICAL SIGNIFICANCE OF D-3-HYDROXYBUTYRATE

There are three main ketones produced as a result of ketosis; D - 3 – Hydroxybutyrate, acetoacetate and acetone.

D-3-Hydroxybutyrate is the most abundant of the three accounting for 75% of total ketones in the body, it is later catabolised into acetoacetate and then into acetone[5]. Due to the higher levels of D-3-Hydroxybutyrate, it is the more sensitive marker for the diagnosis of ketosis, in particular DKA.

D-3-Hydroxybutyrate also has a greater stability compared to either acetone or acetoacetate and is typically stable for 7 days at 4°C. Serious errors can arise if patient analysis is delayed, as such stability is paramount.

FIG 1 KETONE LEVELS CIRCULATING IN THE BLOOD



Measurement of D-3-Hydroxybutyrate is clinically useful in not only the diagnosis of DKA but also in monitoring a patient's response to treatment.

The American Diabetes Association recommends testing for ketosis in diabetics when symptoms of ketoacidosis are present, when glucose levels are consistently elevated, during pregnancy and if experiencing any illness. NICE also recommend monitoring ketones in patients with T1DM especially during periods of illness [6].

3. ADDITIONAL USES OF D-3-HYDROXYBUTYRATE

Ketosis occurs in a number of clinical conditions, the detection of D-3-Hydroxybutyrate therefore plays a vital role.

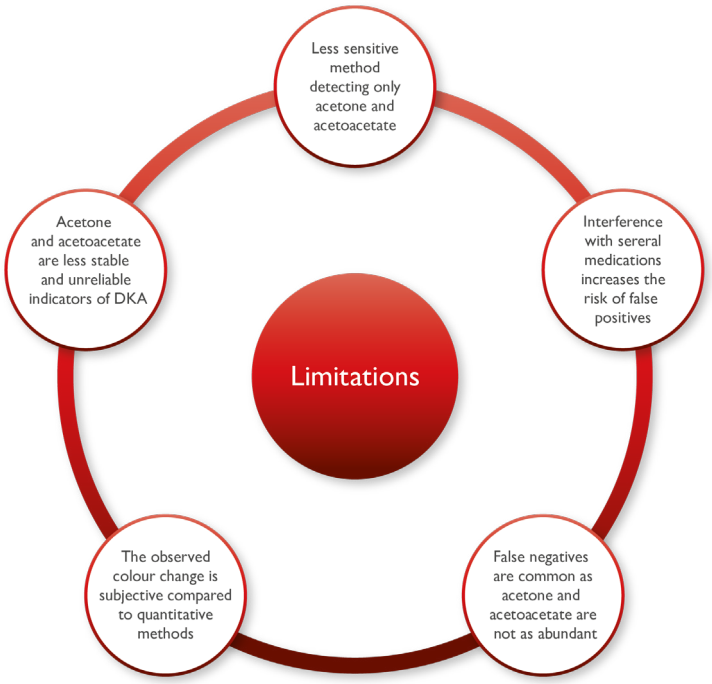
- **Gestational diabetes** – Elevated ketone levels are common in pregnant women with gestational diabetes. If unchecked and untreated these ketone levels can have serious consequences for mother and baby.
- **Alcoholic ketoacidosis** – In Alcoholic ketoacidosis the ratio of D-3-Hydroxybutyrate to acetoacetate is more than twice that in DKA. Monitoring levels of D-3-Hydroxybutyrate is important when making a differential diagnosis between the two conditions.
- **Severe injury, illness or sepsis** - Many critically ill patients respond to starvation by metabolising fats and proteins, which leads to muscle wastage. Monitoring ketone levels in these patients following an overnight fast allows diagnosis of this problem. D-3 Hydroxybutyrate may be used to identify patients likely to benefit from nutritional support.
- **Ketotoic hypoglycaemia** - This refers to any circumstance in which hypoglycaemia is accompanied with ketosis. It is also used to define recurrent episodes of hypoglycaemia with ketosis in young children. Delayed feeding is the most common cause among children with symptoms including vomiting, lethargy, unresponsiveness and even seizures. The detection of D-3 Hydroxybutyrate in the serum or plasma of infants may indicate ketotic hypoglycaemia.
- **Childhood epilepsy** - Regular monitoring of ketone levels may be beneficial in maintaining a ketogenic diet for the control of childhood epilepsy.

4. LIMITATIONS WITH EXISTING METHODS USED TO MEASURE KETONES

Semi-quantitative, nitroprusside-based methods remain common for the detection of ketones in the blood and urine of diabetic patients. The nitroprusside method is available in both tablet and reagent test strip form where urine or blood is applied, and colour change compared to a colour chart. There are several limitations associated with Nitroprusside methods;

- Capable of detecting only acetone and acetoacetate, as such they lack sensitivity especially in early stages of DKA.
- The intensity of the colour change is subjective compared to quantitative methods which can be used to monitor ketosis to resolution and monitor improvements.
- Several medications including Valproic Acid and Vitamin C can interfere with the nitroprusside method leading to false positive results.
- False negative results are common as the method does not detect D-3-Hydroxybutyrate. As ketoacidosis improves and D-3-Hydroxybutyrate is converted to acetoacetate the result becomes positive.
- D-3-Hydroxybutyrate is a more reliable indicator of ketosis and DKA due to its superior stability when compared to acetone and acetoacetate.

FIG 2 – LIMITATIONS WITH METHODS MEASURING KETONES IN URINE



There are additional limitations associated with urine test strips. Blood D-3-Hydroxybutyrate testing is more effective than urine acetoacetate testing in reducing emergency department assessment, hospitilisation and time to recovery from DKA7. Table 1 summarises the advantages of measuring ketones in blood via quantitative clinical chemistry assays compared to semi-quantitative urine test strips.

TABLE 1 – LIMITATIONS WITH METHODS MEASURING KETONES IN URINE

Measuring Ketones in Blood	Measuring Ketones in Urine
Measures D-3-Hydroxybutyrae, the predominant ketone in the blood.	Measures acetoacetate a poor indicator of overall blood ketone levels.
Provides a direct measurement of ketones in circulation.	Provides an indirect measurement of ketones in circulation and is therefore not as accurate.
Indicates current ketone levels allowing immediate medical intervention.	Indicates blood ketone levels in the previous 2-4 hours.
Unaffected by fluctuating fluid levels.	High fluid intake may dilute urine leading to false negatives. Low fluid levels or dehydration may lead to false positives.
Quantitative methods ran on clinical chemistry analysers are more reliable.	Semi-quantitative urine test strips are less reliable and subjective relying on the intensity of the colour change.

5. DIABETIC KETOACIDOSIS IN COVID-19?

Although individuals with diabetes appear to be at similar risk of contracting SARS-CoV-2 the virus that causes COVID-19 they are more likely to suffer severe consequences. There is increasing evidence to suggest that DKA is a common complication in those with Diabetes [8]. DKA was found to both increase the length of stay in hospital and mortality, meanwhile T1DM and T2DM alone had no effect on mortality but did increase hospital stay [9].

6. RANDOX D-3-HYDROXYBUTYRATE (RANBUT) OVERVIEW

D-3-Hydoxybutyrate measurement will enable earlier more reliable detection of ketosis and allow faster treatment. The Randox D-3-Hydroxybutyrate (Ranbut) assay provides a convenient method for the accurate detection of ketones from serum. Suitable for use in open channels, it can be used with a wide range of clinical chemistry analysers.

Key Benefits of the Ranbut Assay

- **Superior methodology** when compared to other commercially available ketone detection tests. For example, the nitroprusside method used in semi-quantitative dipstick tests only detects acetone and acetoacetate. D - 3 - hydroxybutyrate is the most abundant ketone produced during ketosis the measurement of this analyte is more sensitive and specific.
- **Exceptional correlation** coefficient of r=0.9954 when compared against other commercially available methods.
- **Excellent precision** of <3.5% CV.
- **Calibrator and controls available** offering a complete testing package
- **Applications available** detailing instrument specific settings for the convenient use of the Randox D-3-Hydroxybutyrate assay on a wide range of clinical chemistry analysers.
- **Liquid for ease of use.**

7. CONCLUSION

Blood ketone levels provide valuable information to clinicians allowing accurate and reliable identification of DKA. D-3-Hydroxybutyrate as the most abundant ketone in the body is a more sensitive and specific indicator of DKA compared to other available methods on the market. Available for use on a wide range of clinical chemistry analysers, the test is easily automated delivering quantitative results and allowing levels to be monitored for signs of improvement.

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REAGENTS

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