RANDOX

D-3-HYDROXYBUTYRATE AND DIABETIC KETOACIDOSIS

Background

Ketosis is characterised by the ß-oxidation of fatty acids to produce energy in the absence of sufficient levels of glucose in the body. Under normal physiological conditions, energy is produced through glycolysis: the conversion of glucose to pyruvate. However, where there are insufficient glucose levels, ketosis occurs producing several ketone bodies, namely, acetone, acetoacetate, and D-3-Hydroxybuyrate (D-3HB). Ketone bodies are water-soluble molecules produced by the liver in response to ketosis[7]. Overproduction of ketone bodies can result in ketoacidosis. This is most common in diabetic patients who already have complications with the insulin pathway responsible for glycolysis[1].

The severity of diabetic ketoacidosis (DKA) can be ranked mild, moderate, or severe depending on the concentration of ketone bodies present with the most severe cases resulting in death[5]. The mortality rate related to DKA is around 1%, however, this rises to 5% in elderly patients. Other reported causes of DKA include alcohol intoxication, prolonged fasting, and glycogen storage disorders[9].

Ketone bodies are detectable in blood and urine with classical detection methods testing urine for one or more of the ketone bodies characteristic of DKA. Whilst these methods have some utility in ruling out DKA, they are no longer considered diagnostic[2]. New, more robust methods can detect and quantify ketone bodies in serum samples. While this approach can be slightly more invasive, it is a much more accurate predictor of DKA than urine tests[2].

Diabetic Ketoacidosis

DKA is caused by an accumulation of ketone bodies in the body as a result of insulin deficiency occurring most in patients with Type 1 Diabetes Mellitus (T1DM), but an increasing number of cases have been reported in patients with Type 2 Diabetes Mellitus (T2DM) and gestational diabetes[2]. Although most of the patients have a history of diabetes, over 25% of those admitted with DKA were considered to have new onset diabetes[5]. Diagnosis of DKA consists of a high anion gap metabolic acidosis, ketone bodies present in serum and/or urine, and high blood glucose concentration (<250mg/dl)[5,6].

Symptoms of DKA

Patients with DKA may present with the following symptoms:

- Polyuria (excessive urination) and polydipsia (thirst)
- Weight loss
- Fatigue
- Dyspnoea (shortness of breath)
- Vomiting
- Fever
- Abdominal pain
- Polyphagia (excess hunger)
- Fruity smelling breath caused by acetone accumulation

These symptoms are caused by some form of insulin deficiency, for example, new-onset diabetes, insulin non-compliance (not adhering to therapeutic routine) and infections that increase the bodies requirement for insulin[5]. This lack of insulin stimulates the expression of counterregulatory hormones and increases the activity of lipase. This increased activity promotes the liberation of fatty acids from adipose tissue. These free fatty acids are then converted to acetyl co-enzyme A (acetyl CoA) which is subsequently converted to the ketone bodies acetone, acetoacetate and D-3-hydroxybutyrate, otherwise known as ß-hydroxybutyrate[5].

DKA is associated with several complications, the most severe being cerebral oedema. Occurring most frequently in children, this accumulation of fluid in the white matter of the brain has an associated mortality rate of 24% with those who survive at high risk of serious neurological defects[5]. Symptoms of cerebral oedema include headache, persistent vomiting, lethargy, and a host of other neurological symptoms. Other DKA related complications may be hypokalaemia, hypoglycaemia, and acute renal failure[5].

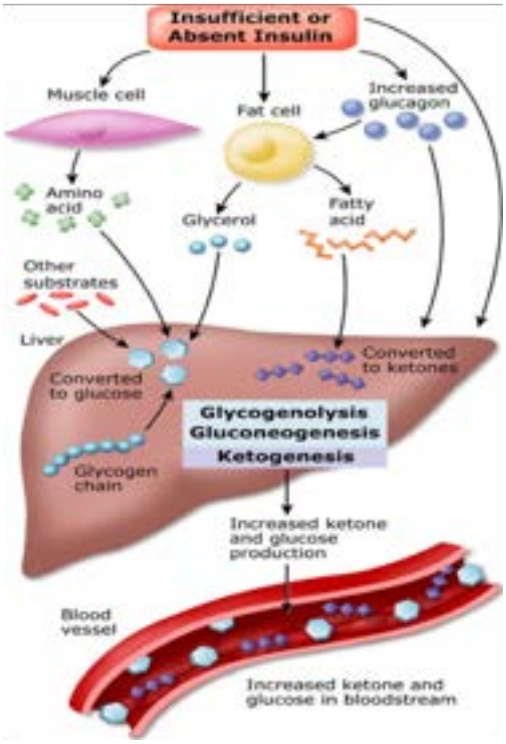

Figure 1. Mechanism of Diabetic Ketoacidosis[12].

Due to insulin deficiency, hepatic and renal glucose production occurs at a higher rate than its metabolism can take place. Once the renal capacity for glucose has been reached, glucosuria and osmotic diuresis cause dehydration. Insulin deficiency stimulates proteolysis, releasing glucogenic and ketogenic amino acids. Finally, insulin insufficiency activates lipolysis. The activation of lipase degrades triglycerides into free fatty acids which are, through glucagon stimulation, oxidised into D-3-hydroxybutyrate and acetoacetate. The subsequent conversion of acetoacetate to acetone provides the three ketone bodies responsible for ketoacidosis.

Euglycemic Diabetic Ketoacidosis

Some patients present with diabetes, high concentrations of ketone bodies and metabolic acidosis but no concurrent elevation in blood glucose levels. This is known as euglycemic diabetic ketoacidosis (EDKA)[6].

EDKA occurs predominantly in T1DM patients but acute illness such as infection, trauma or acute coronary syndrome can trigger EDKA in T2DM patients. Diagnosis of EDKA can prove complicated as increased glucose levels are one of the hallmarks of DKA. Diagnosis of EDKA is one of exclusion. Other causes of anion gap metabolic acidosis, such as sepsis, alcoholic intoxication, drug overdose, lactic acidosis and renal failure, must be ruled out before an EDKA diagnosis can be given[8]. In spite of this, once a diagnosis has been achieved, treatment is relatively straight forward in most cases consisting of insulin administration to alleviate metabolic acidosis and fluids to ameliorate electrolyte imbalance and dehydration[8].

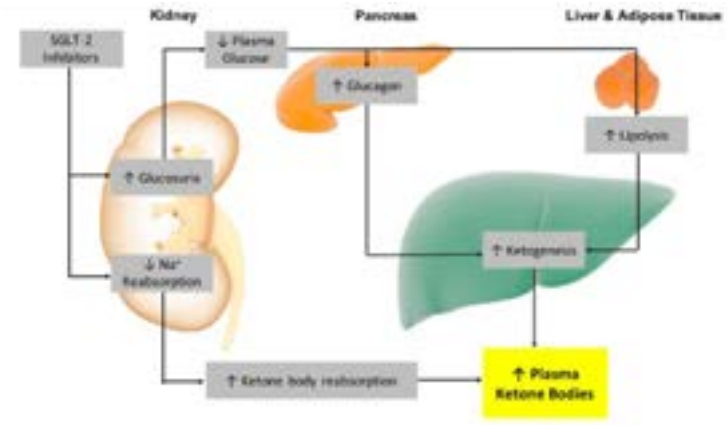

Figure 2. Mechanism of Euglycemic Diabetic Ketoacidosis[13].

Increased lipolysis and fatty acid oxidation as a result of a reduced plasma insulin:glucagon ratio facilitates increased ketone production and renal ketone absorption.

EDKA and Pregnancy

Pregnant women, particularly those with a history of diabetes, are at a higher risk of developing EDKA. The foetal placenta readily consumes glucose to produce energy, resulting in an increase in ketosis. Furthermore, insulin levels are also depressed due to the insulin resistance characteristic of placental and other counterregulatory hormones. These factors mean that pregnant women are constantly battling with glucose starvation and insulin deficiency, increasing the likelihood of ketosis[9].

Ketone Measurement

Classical methods used for the detection of ketone bodies utilise the nitroprusside method. Nitroprusside reacts with the keto-group of acetoacetate and acetone to form a purple-coloured solution. The intensity of the colour can be used for qualitative or semi-quantitative analysis of ketone bodies in a urine sample[7]. This test principle is utilised in urine ketone stick tests which provide an easy and inexpensive method to aid in DKA monitoring and diagnosis. While this test eliminates the need for expensive laboratory equipment and training, urine ketone stick tests detect acetoacetate and acetone but not D-3-Hyrdoxybutyrate, considered to be the predominant metabolite associated with DKA[2].

Figure 3. Colour identification of categories of semi-qualitive urine ketone test sticks[7].

The nitroprusside test has several limitations

- The detection of acetate and acetoacetate in urine is not a true measure of ketone bodies in plasma due to an imbalance between acetone and D-3-Hydroxybutyrate concentrations[5].
- Patients presenting with DKA are often suffering from dehydration, meaning urine volume is significantly decreased. This can delay diagnosis and treatment which may lead to a worse prognosis.
- Urine samples can only provide an average concentration of acetone and acetoacetate in urine since the bladder was last voided.
- The mechanism of treatment for DKA involves the oxidisation of D-3-Hydroxybutyrate to acetoacetate. This can result in a supposed rise in urine ketone bodies, when in fact, the concentration of D-3-Hydroxybutyrate in blood is decreasing, providing a false indication of the success of the treatment[2].
- Urine ketone body concentration is also influenced by other metabolic processes such as kidney function, and some medications[1].
- This test can also experience interference from other substances such as vitamin C.

D-3-Hydroxybutyrate quantification in blood

Modern quantification methods are capable of detecting and quantifying D-3-Hyrdoxybutyrate in a blood sample, rather than urine. These methods allow for rapid and accurate quantification of D-3-Hyrdoxybutyrate present at the time of testing allowing for timely intervention and treatment[2]. The assay principle relies on the oxidation of D-3-Hyrdoxybutyrate to acetoacetate, catalysed by 3-hydroxybutyrate dehydrogenase and the reduction of co-enzyme NAD^+ to NADH. The change in absorbance of the solution is proportional to the concentration of D-3-Hyrdoxybutyrate[1]. One report claims these tests display higher specificity (78%) and positive predictive value (34%) when compared with urine testing (35% and 15% respectively)[5].

Randox D-3-Hydroxybutyrate (Ranbut) Assay

The Randox D-3-Hydroxybutyrate assay is a kinetic enzymatic assay which quantifies D-3-Hyrdoxybutyrate in serum or plasma samples, providing superior test performance when compared with semi-quantitative urine ketone stick tests. The Ranbut assay displays exceptional precision and correlation with other commercially available methods, exhibiting a correlation coefficient of r=0.9954[3]. This assay is available for application on a variety of clinical chemistry analysers and Randox Acusera range includes suitable controls and calibrators, completing the D-3-Hyrdoxybutyrate test package.

One study carried out validation of the Randox D-3-Hydroxybutyrate assay on a Roche Cobas c502 analyser. The authors demonstrate the excellent precision of this assay, reporting interlaboratory coefficient of variation of 1.5% and 6.8% for high and low concentration samples respectively[1]. This report stated that the Randox D-3-Hydroxybutyrate assay was to be considered analytically superior to any semi-quantitative urine ketone testing, yielding highly reliable results in the clinically relevant ranges, providing laboratories with a precise and reliable diagnostic tool for the detection and monitoring of ketoacidosis[1].

Table 1. Comparison of median D-3-Hydroxybutyrate plasma concentrations among five semi-qualitative urine ketone categories. Displays considerable overlap between semi-qualitative categories[1].

Category	N	Median (IGR)	Range
negative	46	0.06 (0.02-0.18)	0.00-0.69
+	40	0.53 (0.13-0.75)	0.00-2.42
++	40	0.95 (0.60-1.50)	0.22-3.21
+++	40	1.49 (0.70-3.06)	0.05-6.82
++++	24	2.77 (2.04-5.60)	0.53-10.53
diab. ketoacid.	11	5.72 (3.85-7.59)	3.34-10.53

Conclusions

DKA is characterised by high anion gap metabolic acidosis, the presence of ketone bodies in blood and urine, and high blood glucose concentration. EDKA is more difficult to detect due to its distinctive lack of elevation of blood glucose concentration. Classic urine detection methods are subject to several limitations which hinder its utility in DKA, and particularly EDKA diagnosis. Methods which detect D-3-hydroxybutyrate in blood samples, such as Randox D-3-Hydroxybutyrate assay, provide a more accurate and reliable procedure for the quantification of ketone bodies and the diagnosis of DKA.

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