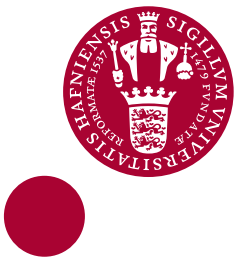

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Death from diabetic ketoacidosis in the Eastern part of Denmark in 2016-2018. Beta-hydroxybutyrate as a marker

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Abstract:

Diabetes mellitus is a disease caused by a deficiency in (type 1) or inability to use insulin (type 2). Untreated it can lead to diabetic ketocidosis (DKA) – state with high levels of ketone bodies (acetone, acetoacetate, beta-hydroxybutyrate (BHB)). This state can be life threatening. Measurement of ketone bodies together with vitreous/urine glucose and glycosylated hemoglobin (HbA1C) are therefore essential to diagnose DKA-related deaths.

All autopsy samples received at our department in the period 2016-2018 for toxicological investigations were analyzed for acetone, BHB, and vitreous glucose (N = 1394). In case of a high level of BHB, HbA1C and urine glucose were measured. Thirty two cases (2.3%) were concluded to be DKA-related deaths. Eleven (34%) of these had no known history of diabetes.

BHB accounts for the major part of ketone bodies and is directly associated with the acidosis effect. Therefore, BHB is preferred to acetone when evaluating DKA and other ketoacidosis-related deaths. We compared acetone and BHB levels to evaluate if the easy acetone measurement could cover our needs for screening. We found that high BHB levels (>2000 µmol/L) were detected if the acetone cut off was set to 0.01 g/L. But, many samples would have low BHB < 3-500 µmol/L with this cut off, and many samples with raised BHB (500-1,200 µmol/L) would not be detected. We therefore recommend to screen all samples for BHB. In case of a high BHB (>1,000 µmol/L) vitreous/urine glucose and HbA1C must be measured to distinguish DKA from other types of ketoacidosis.

Keywords:

Diabetic ketoacidosis, BHB, HbA1C, vitreous glucose, postmortem blood

INTRODUCTION

Some cases of unexpected death that may be subjected to forensic investigation are caused by newly arisen or mal-treated diabetes

In untreated insulin-demanding diabetes the body becomes dependent of fatty acid metabolism for energy. Concurrently, an overproduction of ketone bodies (acetone, acetoacetate, BHB) takes place, which indicates a state of acidosis that may lead to coma and death. BHB is in fact not a ketone but is considered as a ketone body because it is in an equilibrium with acetoacetate.

Diabetes mellitus occurs as either a deficiency in (type 1) or inability to use insulin (type 2). In 2017, a total of 252,750 people in Denmark had the diagnosis, diabetes [1]. The vast majority, about 80-90%, had type 2-diabetes. It was estimated that 60,000 Danish people (5%) did not know that they had type 2-diabetes. The number of individuals with diabetes have more than doubled from 2000 to 2016 [1]. The total number of deaths among diabetic patients in Denmark was 11.901 in 2011 [2]. Of these, 7.237 had major complications of their diabetes [2]. Not only lifestyle but also mental illness like schizophrenia are associated with occurrence of a diabetic state [3]. The background is that antipsychotic drugs like olanzapine, clozapine and possibly quetiapine are associated with the risk of diabetes due to the induction of overweight [3].

In living subjects, diabetes is diagnosed from fasting plasma glucose ≥ 126 mg/dl [4] and HbA1C ≥ 45.5 mmol/mol [5]. Other signs like polyuria, weight loss, ketonemia and ketonuria are also included in the evaluation of diabetes. Glucose and HbA1C are useful markers of an individual glycemic state [6]. Blood glucose is a snapshot of the glycemic state at the time of blood sampling. It can vary depending on food intake, stress etc. [6]. In comparison, HbA1C is useful in glucose control as it reflects the average blood glucose in 2-3 months and is free of fluctuations in glucose concentrations [6]. HbA1C or glycated hemoglobin is formed when glucose is added non-enzymatically to hemoglobin. The amount of HbA1C is compared to total hemoglobin and gives an expression of the mean blood glucose level for the previous 2 months, which is the lifespan of the reds blood cells [7]. HbA1C has gained increased interest in postmortem diagnosis of diabetes because it is a stable and a useful marker of the ante-mortem blood glucose level within 2 month [7-9]. Due to a rapid decrease in blood glucose concentration after death, blood glucose cannot be used for reliable identification of diabetes in postmortem cases. Instead, vitreous glucose and/or urine glucose, if these are measured within few hours after sampling from autopsy, can be used together with ketone bodies in blood (acetone and BHB) and HbA1C as diagnostic tools. Palmiere [10] recommends BHB as the best choice for detecting ketone bodies. Measurement of glucose in vitreous fluid and urine together with HbA1C are important to diagnose diabetes as the cause of ketoacidosis.

The aim of this study was to evaluate cases with DKA as a cause of death in a three-year period from 2016 to 2018. We were especially interested in studying DKA deaths without a history of diabetes mellitus. In addition, it was evaluated whether acetone could replace BHB as an indicator of diabetes as acetone is easily analyzed together with ethanol on headspace gaschromatography.

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EXPERIMENTAL PROCEDURES

Whole blood samples (femoral), vitreous fluid and urine collected from autopsy cases received for toxicological investigation at the department of forensic chemistry in Copenhagen in 2016-2018 were analyzed. The department performed 2068 autopsies in the three-year period. A toxicological investigation was performed in 1394 of the autopsy cases. Glucose was measured in vitreous fluid and urine. Whole blood was used for determination of HbA1C, acetone and BHB. In two cases with limited amount of blood, BHB was analyzed in muscle tissue.

The vitreous fluid and urine glucose concentrations were analyzed by an amperometric measurement by use of an ABL 90FLEX instrument (Radiometer). Cases with levels > 5 mmol/L in vitreous fluid and/or urine were considered for further consideration.

HbA1C was measured by a combination of immunology and spectrometry technique using a DCA Vantage Analyzer (Siemens). Values below 42 mmol/mol (<6%) were considered as normal. Values from 42 mmol/mol up to 75 mmol/mol (6-9%) were referred to as controlled diabetes, and higher values as uncontrolled diabetes.

BHB together with GHB (gammahydroxy butyric acid) were analysed by an in-house developed method that included a precipitation extraction step followed by UHPLC-MS/MS [11]. Blood BHB levels < 300 µmol/L were considered as normal. Blood levels above 300 to 1,000 µmol/L as raised. Blood levels above 1000 µmol/L were defined as significantly raised, and blood levels > 2,000-3,000 µmol/L as indicative of ketoacidosis [12].

Acetone was determined as part of the routine ethanol analysis (BAC). An Agilent gas chromatography system equipped with a 6890 flame ionization detector (GC-FID) and a G1888 head-space sampler was used for the determination. A sample volume of 100 µl (whole blood) was sampled and analysed on a Restek capillary columns (Rtx-BAC1). The internal standard was 2-Butanol. The chromatography was isothermal at 40^{0}C, with nitrogen as the carrier gas.

External quality programs were followed covering acetone and HbA1C. The laboratory is accredited according to ISO 17025.

As this is a retrospective study on data routinely collected by the laboratory, the survey was not reported to the National Committee on Health Research Ethics in Denmark.

RESULTS

In total 32 DKA cases were observed in the years 2016-2018. Eleven (34%) of the DKA deaths had no known history of diabetes. In the following called "unknown DKA".

Table 1 gives an overview of all DKA deaths. All the unknown diabetes deaths were middle-aged men, median age 45 with range 31-63, while 33% of the known DKA deaths were women. The group of known diabetes deaths were also of middle age, but the age range was wider and the median age was 10 years higher. HbA1C tended to be higher for the unknown DKA cases than for the known cases, where treatment probably might have reduced blood glucose, although not preventing deaths. The range of HbA1c was 53->130 mmol/mol in the known diabetic group and 93->130 mmol/mol in the unknown DKA. Exposure to cold could have influenced the elevated BHB level (ketone bodies) in one case with low HbA1C (53 mmol/mol), Table 1.

The eleven cases of undiagnosed diabetes with DKA as cause of death are presented in Table 2. Four suffered from schizophrenia and a fifth was positive for quetiapine. As quetiapine is used for mental illness like schizophrenia, at least 45% of the unknown diabetics were diagnosed with schizophrenia or other psychiatric illness. In two other cases of the unknown DKA deaths there was information about anxiety or mental fragility. While looking through the cases with known diabetes, one schizophrenia case and two cases with antipsychotic treatment (olanzapine and quetiapine respectively) were observed. This indicates that 14% in the group of known diabetics had schizophrenia or similar illness. Four (19%) of the known diabetics were diagnosed with type 1 diabetes and nine (42%) had type 2 diabetes. Further, three was suspected to have type 1 diabetes and three were suspected to have type 2 diabetes. It was not possible to differentiate between type1 and type 2 in two cases.

The correlation between acetone and BHB for all cases analyzed in the period is shown in Figure 1. An acetone cut off of 0.01 g/L includes all cases with blood BHB above 2000 µmol/L but many cases with raised blood BHB from 500 to 1200 µmol/L will not be detected. Moreover, several cases had blood BHB values below 300-500 µmol/L, while acetone was above 0.01 g/L.

Table 1.

	All cases	Known diabetes	Unknown diabetes
Number	32	21	11
Gender	78% male	67% male	100% male
Age range	29 - 80	29 - 80	31 - 63
Median age	48.5	54	45
Vitreous humor, glucoseRange mmol/L	5.2-77	5.2-77	25 – 73
Vitreous humor, glucoseMedian mmol/L	34	32.5	34
Blood BHB range (µM)*	740 – 21,000	740 – 21,000	1,400 – 13,000
Blood BHB median (µM)	9,550	6,600	9,000
HbA1C range (mmol/mol)	53 - >130	53 - >130	93 - >130
HbA1C median (mmol/mol)	**116**	**93**	**>130**

* BHB was analyzed in muscles tissue in two cases.

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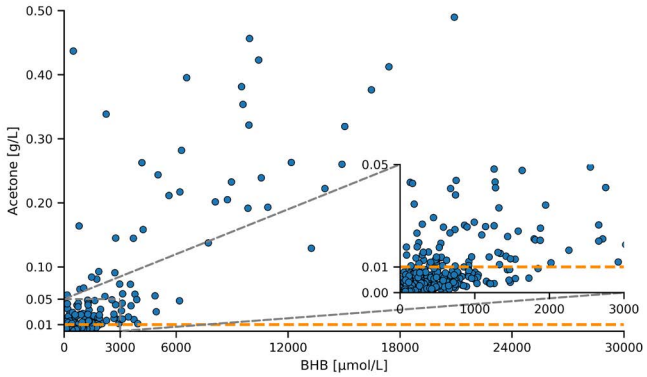

Figure 1

DISCUSSION

Although, this study presents a limited data set on DKA related deaths, it is in agreement with findings elsewhere [13]. Ali et al [13] found that about a third of all DKA deaths in a 6 year period were unknown diabetes. This underlines the importance of having tools to discover these cases. It is important to measure vitreous glucose and HbA1C along with blood BHB/acetone to distinguish DKA from other types of ketoacidosis. Ketoacidosis can quickly develop in chronic alcoholics. Blood alcohol will be low or absent is these cases [14;15]. Because of malnutrition and inhibition of ethanol on glucose production blood glucose will be low to normal and ketone bodies (BHB/acetone) will be high [14-17]. Cold exposure and starvation are states that also are associated with ketoacidosis due to increased breakdown of fatty acids [14;16;18]. Blood glucose will be normal or low. Because HbA1C is an expression of blood glucose within a 2 months period, HbA1C will be normal in these examples. An overview of different states of ketoacidosis is given in Table 3. The hyperosmolar hyperglycemic state is included in Table 3, even though it is not associated with ketoacidosis. The hyperosmolar state most often occurs in type 2 diabetes with reduced fluid intake. Hyperglycemia leads to dehydration because of an increased diuresis [17].

Ali et al. [13] also found a higher prevalence of males in the unknown than in the known diabetes DKA deaths. The unknown diabetes DKA deaths in our study consisted solely of men.

Diabetes mellitus appears to be more common in schizophrenic patients than in the general population [3]. The antipsychotic drugs clozapine and olanzapine have been shown to predispose to diabetes mellitus because of induction of overweight [3]. The higher prevalence of mental illness like schizophrenia and anxiety observed among the unknown diabetes DKA cases compared to those with known diabetes

Table 2.

Year	Gender	Age	HbA1Cmmol/mol	GlucoseVitreous humormmol/L	BHB in blood µM	Psychiatric diagnosis	Antipsychotic treatment	Toxicological findings
2016	male	40	>130	nt	9,500	Schizophrenia	Unknown	Negative
2016	male	56	>130	43	2,200	Schizophrenia	Risperidon	Risperidone in therapeutic conc.
2016	male	63	93	nt	1,400	-	-	Negative
2017	male	39	>130	27	11,000	-	-	Quetiapine, Acetaminophen
2017	male	44	>130	65	9,000	-	-	Negative
2017	male	47	>130	nt	13,000	-	-	Salicylic acid
2018	male	31	>130	34	8,000*	-	-	Negative
2018	male	48	>130	32	11,000	Schizophrenia	No treatment	Negative
2018	male	55	119	nt	3,400*	-	-	nt for drugs
2018	male	42	>130	25	10,000	Schizophrenia	No treatment	Negative
2018	**male**	**45**	**>130**	**73**	**8,100**	**-**	**-**	**Negative**

nt: not tested; *: Analyzed in muscles tissue

Table 3.

	Blood Alcohol Promille	Acetone	Beta-hydroxybutyrate	Vitreous glucose	Urine Glucose	Blood Glucose	HbA1C
Diabetic ketoacidosis	-	High	High	High	High	High	High
Hyperosmolar glycemic state	-	Low	Low	High	High	High	High
Alcoholic ketoacidosis	Low or zero	High	High	-	-	Low or zero	Normal
Cold exposure	-	High	High	-	-	Normal	Normal
Starvation	**-**	**High**	**High**	**-**	**-**	**Low**	**Normal**

indicates a barrier towards reacting on symptoms and seeking a doctor. Furthermore, physicians should be aware that patients treated with antipsychotics like clozapine and olanzapine have a risk of developing overweight and thereby diabetes.

About 78% of the total ketone bodies consist of BHB while acetone only accounts for 2% [10;17]. Moreover, acetone does not contribute directly to an acidosis [17]. But acetone is formed from acetoacetate by decarboxylation producing carbonic acid. As the body can produce acetone after death, BHB is considered the best choice for ketone determination [10]. Some authors recommended to screen for acetone instead of BHB because acetone is easily detected along with ethanol [16;17;19]. Sadones et al. [19] retrospectively analyzed 599 cases for BHB and found that ketone determination could be limited to acetone. In our study, we found that all cases with a high BHB (BHB > 2000 µmol/L) were detected with an acetone cutoff of 0.01 g/L. But, we lost many cases with an elevated BHB level (500 to 1200 µmol/L), and many cases would be included even if BHB was below 500 µmol/L. The latter would cause a lot of extra work to reveal an eventual acidosis. We therefore recommend that BHB as the first choice. We have set up an UHPLC-MS/MS method that routinely and easily measure GHB (gammahydroxybutyric acid) and BHB in all living and autopsy cases. Another argument for preferring BHB to acetone has been that an accidently or intentionally intake of acetone or isopropanol before death could influence the result [17;19]. However, BHB is also an alternative energy source in the body. This is especially important in starvation when glucose dependent tissue use BHB instead of glucose as an energy source [20]. The alternative medicine industry has focused on BHB`s energy effect, and the compound is sold as a "health" product, which might provide a differential diagnostic problem in some cases [21].

A ketogenic diet (low intake of carbohydrates) is also associated with hyperketonemia and metabolic acidosis [18]. Therefore, to evaluate suspected cases of DKA and to distinguish between different kinds of acidosis we recommend to measure vitreous/urine glucose and HbA1C along with BHB. All blood samples should be screened for BHB/acetone to discover DKA deaths in undiagnosed diabetics. In case of high levels of BHB (>1000 µmol/L) vitreous/urine glucose and HbA1C should be measured.

CONCLUSION

BHB can easily be included in the routine analysis with only a little extra work. To avoid losing important information on cause of death we recommend to investigate all autopsy cases for blood BHB. In case of high levels (>1000 µmol/L) vitreous/urine glucose and HbA1C should be measured to differentiate between the different kinds of ketoacidosis.

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