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Characteristics of post-mortem beta-hydroxybutyrate-positivet cases – A retrospective study on age, sex and BMI in 1407 forensic autopsies

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ABSTRACT

Background: Post-mortem biochemistry, including the analysis of beta-hydroxybutyrate (BHB), is increasingly employed in forensic medicine, especially in conditions such as diabetes and chronic alcoholism. However, not much is known about the associations between age, body mass index (BMI), and sex and BHB concentrations in ketoacidotic conditions.
Aim: To retrospectively study the association between age, BMI and sex in several conditions, such as diabetic ketoacidosis (DKA), alcoholic ketoacidosis (AKA), and elevated *post-mortem* BHB concentrations.
Methods: 1407 forensic autopsy cases analysed for BHB were grouped by diagnosis: DKA, AKA, HHS [hyperosmolar hyperglycaemic state], acidosis NOS [not otherwise specified], or hypothermia. Age, sex, BMI and the concentrations of blood alcohol, vitreous glucose and blood BHB were recorded.
Results: Cases of AKA and DKA were most numerous (184 and 156, respectively). In DKA and in its male subgroup, cases with severe ketosis (BHB > 1000 μg/g) were younger and had a lower BMI than those with moderate ketosis (BHB 250–1000 μg/g) and controls ($P < 0.001$). In DKA and in its female subgroup, cases with moderate ketosis cases were older ($P = 0.0218$ and $P = 0.0083$) than controls. In AKA and in its male subgroup, cases with severe ketosis had a lower BMI than those with moderate ketosis ($P = 0.0391$ and $P = 0.0469$) and controls ($P < 0.001$). Cases with moderate ketosis had a lower BMI than controls ($P < 0.001$).
Conclusions: BHB concentration is associated with BMI in DKA and AKA, and with both BMI and age in DKA. Constitutional factors should, therefore, be considered in potential AKA and DKA cases.

1. Introduction

Ketoacidosis refers to a state of metabolic imbalance that may develop when the glucose metabolism is impaired and fatty acids are subsequently released from adipocytes. Ketoacidosis may develop due to numerous conditions. In the forensic context, diabetes, alcohol abuse, starvation, and hypothermia are the most frequently encountered conditions [1]. In ketogenesis, mainly beta hydroxybutyrate (BHB), acetoacetate and acetone are formed. Of these, the concentration of BHB rises the fastest in the ketogenetic process [2].

A BHB concentration greater than 250 μg/g blood is viewed pathological. In forensic cases in which such a BHB concentration is encountered, ketoacidosis can be considered to be the immediate or underlying cause of death [3–5], after other causes of deaths have been excluded. Among living subjects, a concentration up to 50 μg/g is considered normal. A concentration above 100 μg/g is defined as hyperketonemia and above 300 μg/g as ketoacidotic [6].

Previous research on *post-mortem* BHB has focused primarily on the diagnosis of ketoacidosis and on suggesting a cause: either diabetes (diabetic ketoacidosis, DKA) or alcohol (alcoholic ketoacidosis, AKA) [4,5,7]. Less attention has been paid to other factors, such as associations between BHB concentration and age or sex. Differences in the prevalence of DKA between the sexes have been investigated in patients, with conflicting results. Some studies have shown a female preponderance [8,9], while others a male one, at

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least in certain subgroups [9]. Age, on the other hand, is associated with a higher mortality in DKA [10]. As far as we know, no studies have been made on the impact of age and sex on AKA.

Obesity is associated with type 2 diabetes and plays a role in some cases of type 1 diabetes [10]. Chronic alcoholics, on the other hand, are often malnourished [11] and there is a negative correlation between body mass index (BMI) and alcohol consumption, at least among women [12,13]. Lean et al. reported that obesity and total alcohol consumption are associated, while the frequency of drinking alcohol is negatively associated with obesity [14]. Yokoyama et al. reported that low BMI is associated with AKA in a study of alcoholic men [15]. There is also evidence that in type 2 diabetics, DKA develops in older and obese patients [16]. The association between age, sex and BMI and different types of ketoacidosis has not been investigated in a *post-mortem* context.

Uncontrolled diabetes can lead to hyperglycaemic emergencies such as DKA or a hyperosmolar hyperglycaemic state (HHS). An HHS typically develops in type 2 diabetics who suffer from an acute stressor, for example an infection. The acute stressor worsens the blood glucose balance, but the body is still able to suppress the acidosis. Hence, hyperglycaemia without concomitant ketoacidosis develops [5,17]. Mortality in HHS is higher than it is in DKA [18]. Hockenhull et al. identified six cases of a total of 102 deaths that had an elevated glucose concentration without significantly elevated concentrations of ketone bodies, which indicated possible HHS [5]. No other study has examined the occurrence of HHS *post mortem*.

We have therefore retrospectively investigated the association between age, BMI and sex in *post mortem* cases analysed for BHB with special focus on AKA and DKA deaths. We also estimated the proportions of various diagnoses that have been associated with elevated BHB concentrations.

2. Materials and methods

2.1. Case selection

All cases analysed for BHB in the autopsy routine at the National Board of Forensic Medicine in Sweden during the years 2013–2018 were retrospectively included in this study, giving 1490 *post-mortem* cases. Children were excluded (nine cases under the age of 18 at the time of death), as were also 74 cases in which another tissue than femoral blood had been analysed. Thus, 1407 cases remained, of which 1061 were men and 346 women.

During the study period, the BHB concentration was measured at the request of the forensic pathologist or the forensic toxicologist. Alcohol was analysed in all forensic *post-mortem* toxicological cases as long as there was enough substrate for an analysis.

2.2. Analytical methods

BHB concentrations were determined using a 6890 N gas-chromatograph (GC) coupled to a 5973 mass-spectrometer (Agilent technologies). 250 mg of blood was mixed with 100 µL internal standard solution (BHB-D4), and 50 mL of 0.2 M sulphuric acid. 1 mL of ethyl acetate was added. The solution was incubated for three minutes before centrifugation. The supernatant was transferred and evaporated under nitrogen until it reached dryness. The analytes were reconstituted in 100 µL ethyl acetate and 50 µL BSTFA + 1% TMCS (Thermo Scientific). After 30 min of derivatisation at 60 °C, and 10 min of cooling, 2 µL were injected on the GC with a 250 °C injection temperature. The analytes were separated on a DB-5MS (95% dimethyl - 5% diphenyl polysiloxane) column. The temperature gradient was elevated from 70 °C to 110 °C in 4 min and then held for 0.2 min. Subsequently, the temperature was elevated by 40 °C per minute until it reached a level of 315 °C, after which the temperature was held for 1 min. A flow of 1.0 mL of helium per minute was added during the process. A standard curve from 50 to 1000 µg/g was used for quantification and the quantifier ions were 237 *m/z* for BHB-D4 and 233 m/z for BHB. The cut-off level of BHB was 50 µg/g and the upper detection limit was 1000 µg/g. BHB concentrations < 50 µg/g were reported as negative and BHB concentrations > 1000 µg/g as BHB > 1000 µg/g. The precision of the method, measured as coefficient of variance, was <5% and the accuracy (interday and intraday accuracy, n = 8 at three different concentrations) was between 93% and 100%.

2.3. Cause of death

Autopsy protocols, background information, results from ancillary investigations and medical records (when available) were evaluated, and the cause of death was determined from these.

Based on the forensic pathologist statement and the information available the included cases were divided into ten different groups according to cause of death: *AKA cases* – AKA was considered cause of death and the BHB concentration ≥ 250 µg/g, *DKA cases* – DKA was considered cause of death and the BHB concentration ≥ 250 µg/g, *AKA not CoD* – AKA was present but not considered cause of death, *DKA not CoD* – DKA was present but not cause of death, *Hypothermia* –hypothermia was considered cause of death, *Hypothermia contributing*–hypothermia was considered a contributing cause of death, *HHS cases* – HHS was present (glucose was elevated but the BHB concentration was negative or below 250 µg/g) and considered cause of death, *Acidosis NOS CoD* – ketoacidosis NOS (not otherwise specified, *i.e.*, other than DKA, AKA or hypothermia) was present (BHB concentration ≥ 250 µg/g) and considered cause of death, *Acidosis NOS not CoD* – ketoacidosis NOS was present (BHB ≥ 250 µg/g) but not considered cause of death, *Negative or other CoD* – the remaining cases where the BHB concentration was negative or below 250 µg/g or had another CoD. An overview of the cause of deaths and further subdivision of the cases can be found in Fig. 1.

Cases with elevated BHB and vitreous glucose concentration were defined as DKA. Individuals with DKA who had diabetes type 1 and those with type 2 were grouped together. Cases with known diabetes and elevated BHB without elevated glucose were classed as DKA, since *post-mortem* DKA can exist without an elevated glucose concentration [19]. Cases with elevated BHB and known alcoholism were defined as AKA. The existence of chronic alcoholism was most often established from a comment about long-time alcohol abuse in the police report or in medical records, and, in a few cases, from *post-mortem* biochemical analysis (the phosphatinylethanol, PEth,

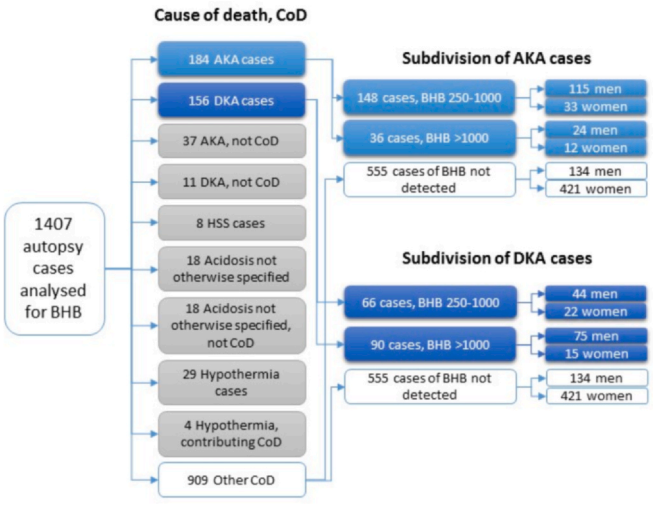

Fig. 1. Flow chart showing the cause of death group and the subdivision of the AKA and DKA cases for further analysis.

Table 1
Diagnosis and the corresponding variable characteristics.

	Number of cases	% of cases	Age (y), median (range)	% women (n)	% BHB 50–249 µg/g (n)	% BHB 250–1000 µg/g (n)	% BHB >1000 µg/g (n)	BMI (kg/m^2), median (range)	Blood alcohol (‰), median (range)	Vitreous glucose (mmol/L), median (range)
AKA, CoD	184	13.1%	64 (37–85)	24.5% (45)	0%	80.4% (148)	19.6% (36)	22.2 (10.7–40.6)	0.57 (<0.1–3.05)	0.6 (<0.2–17.1)
DKA, CoD	156	11.1%	58 (19–92)	23.7% (37)	0%	42.3% (66)	57.7% (90)	22.7 (12.2–44.8)	0.40 (<0.1–1.65)	34.2 (<0.2–78.4)
AKA, not CoD	37	2.6%	62 (32–78)	18.9% (7)	0%	94.6% (35)	5.4% (2)	24.2 (20.5–31.2)	0.62 (<0.1–1.81)	7.7 (0.4–50.3)
DKA, not CoD	11	0.7%	58 (33–85)	9.1% (1)	0%	81.8% (9)	18.2% (2)	24.1 (13.6–38.7)	0.72 (<0.1–3.56)	0.75 (<0.2–22.4)
HHS, CoD	8	0.5%	62 (37–85)	50.0% (4)	25.0% (2)	0% (0)	0% (0)	28.8 (22.3–45.5)	1.63 (<0.1–2.37)	24.0 (11.3–75.6)
Acidosis NOS, CoD	18	1.3%	66 (38–78)	38.9% (6)	0%	83.3% (15)	16.7% (3)	19.4 (10.9–34.9)	0.62 (<0.1–1.20)	0.5 (<0.2–0.9)
Acidosis NOS, not CoD	51	3.6%	70 (22–96)	31.4% (16)	0%	96.1% (49)	3.9% (2)	22.1 (10.9–42.4)	0.26 (<0.1–0.54)	0.4 (<0.2–7.3)
Hypothermia, CoD	29	2.1%	64 (21–92)	34.5% (10)	20.7% (6)	72.4% (21)	6.9% (2)	24.2 (15.2–35.9)	0.76 (<0.2–1.85)	0.85 (<0.2–25.2)
Hypothermia, contributing CoD	4	0.3%	68 (56–77)	25.0% (1)	75.0% (3)	25.0% (1)	0% (0)	26.0 (18.9–31.0)	<0.1 (<0.1)	1.5 (0.4–3.3)
Other CoD/no acidosis	909	64.6%	61 (18–95)	24.1% (219)	39.5% (359)	0%	0%	25.0 (9.9–61.9)	0.97 (<0.1–4.81)	0.7 (<0.2–52.8)
Total	1407	100%	62 (18–96)	25.0% (346)	26.3% (370)	24.6% (346)	9.8% (138)	24.0 (9.9–61.9)	0.72 (<0.1–4.81)	0.7 (<0.2–78.4)

Note: AKA – alcohol ketoacidosis, CoD – cause of death, DKA – diabetes ketoacidosis, HHS – hyperosmolar hyperglycaemic state, NOS – not otherwise specified, *i.e.*, acidosis for other or for unknown reasons. Median and range as well as percentages were calculated based on available data.

concentration ≥ 0.7 µmol/L in femoral blood [20]). As HHS were labelled cases which had high enough vitreous glucose values (≥10 mmol/L) to be lethal [21,22], but in which BHB was not detected or below 250 µg/g.

We chose a cut-off concentration for BHB of 250 µg/g, to ensure that only cases that were truly pathologically ketoacidotic [3–5] were evaluated. In some cases, an elevated BHB concentration could be explained by one of several reasons. In such cases, the ketoacidosis was classified according to the most probable reason for the acidotic state, with both glucose concentration and background information being considered. In cases in which alcoholism and/or diabetes had been diagnosed, the ketoacidosis was labelled according to one of these, even if other possible causes, such as an acute infection, were present. We assumed that the underlying diabetes or alcoholism had played a role in the development of the ketoacidosis in these cases [23]. However, when the BHB elevation was assumed to be due to hypothermia, the case was labelled as hypothermic, even if alcoholism and/or diabetes had been diagnosed, as acidosis may develop independently in hypothermia [24]. Some cases of acidosis lacked sufficient background information and other findings to allow them to be grouped as AKA, DKA, HHS or hypothermia. These were labelled acidosis not otherwise specified (NOS).

Sex, age at the time of death, BMI and the results of blood alcohol and vitreous glucose analyses for the 1407 study cases were retrieved from the National Board of Forensic Medicine database. The lower detection limit for blood alcohol was 0.1‰ and the upper detection limit was 6.0‰. The lower detection limit for vitreous glucose was 0.2 mmol/L and the upper detection limit was 38.9 mmol/L. If a higher concentration was encountered, the samples were diluted.

2.4. AKA and DKA analysis, and grouping based on BHB concentrations

In addition to the separation according to diagnosis, the 1407 cases were also separated according to their BHB concentrations, regardless of underlying condition, into the following groups: BHB not detected (BHB < 50 µg/g), BHB positive (BHB 50–249 µg/g), moderate ketoacidosis (BHB 250–1000 µg/g) and severe ketoacidosis (BHB > 1000 µg/g). The median age, BMI, blood alcohol concentration, vitreous glucose concentration and proportion of women were noted or calculated for all these groups.

The DKA and AKA groups for which the ketoacidosis had been deemed cause of death were investigated in more detail. These groups were also divided according to the degree of ketoacidosis, see Fig. 1. Cases with BHB 250–1000 µg/g were labelled moderate DKA or AKA and cases with BHB > 1000 µg/g as severe DKA or AKA. The cases were also separated into men and women, see Fig. 1. The parameters listed above were also recorded for these groups. The cases in which BHB was not detected (BHB < 50 µg/g) were used as control group.

2.5. Statistical analysis

Data were analysed using the GraphPad Prism version 8.3.0 software (GraphPad Software, Inc). The significances of differences in median age and BMI between cases with different BHB concentrations were tested using the Kruskal-Wallis test with Dunn's adjustment for multiple comparisons. The differences in the fraction of cases and sex distribution between groups were investigated using the Chi squared test. A P-value below or equal to 0.05 was considered statistically significant.

3. Results

Of the total number of cases, 60.7% (854) were positive for BHB (BHB ≥50 µg/g) and 34.2% (481) of the total number of cases were ketoacidotic (BHB ≥ 250 µg/g), see Table 1 and Supplementary Table 1. Of the ketoacidotic cases, the most common cause of death

Table 2
Diabetes ketoacidosis cases, cause of death and controls.

		Number of cases	% of cases	% women (n)	Age at time of death (y), median (range)	BMI (kg/m^2), median (range)	Blood alcohol (‰), median (range)	Vitreous glucose (mmol/L), median (range)
Total	BHB negative	555	39.4%[a]	24.1% (134)	60 (18–95)	25.7 (9.9–61.9)	1.18 (<0.1–4.81)	0.8 (<0.2–75.6)
	All DKA cases	156	100%	23.7% (37)	58 (19–92)	22.7 (12.2–44.8)	0.40 (<0.1–1.65)	34.2 (<0.2–78.4)
	BHB 250–1000 µg/g	66	42.3%	33.3% (22)	66 (19–82)	26.4 (16.0–44.8)	0.42 (<0.1–1.65)	18.9 (0.3–71.0)
	BHB > 1000 µg/g	90	57.7%	16.7% (15)	53 (19–92)	21.5 (12.2–33.0)	0.23 (<0.1–1.09)	44.8 (<0.2–78.4)
Women	BHB negative	134	24.1%[b]	100%	57 (19–90)	24.6 (9.9–56.7)	1.56 (<0.1–4.81)	0.7 (<0.2–52.8)
	All female DKA	37	100%	100%	62 (34–82)	22.7 (13.3–44.8)	0.33 (<0.1–0.66)	29.7 (<0.2–71.0)
	BHB 250–1000 µg/g	22	59.5%	100%	66 (34–82)	24.4 (16.0–44.8)	0.33 (<0.1–0.66)	20.2 (0.3–71.0)
	BHB > 1000 µg/g	15	40.5%	100%	58 (34–80)	20.8 (13.3–29.4)	0.30 (<0.1–0.46)	36.05 (<0.2–58.3)
Men	BHB negative	421	75.9%[b]	0%	61 (18–95)	26.0 (13.6–61.9)	1.05 (<0.1–4.73)	0.8 (<0.2–75.6)
	All male DKA	119	100%	0%	57 (19–92)	22.7 (12.2–39.2)	0.40 (<0.1–1.65)	37.95 (<0.2–78.4)
	BHB 250–1000 µg/g	44	37.0%	0%	64 (19–81)	26.8 (19.4–39.2)	0.42 (<0.1–1.65)	18.35 (0.30–60.6)
	BHB > 1000 µg/g	75	63.0%	0%	52 (19–92)	21.7 (12.2–33.0)	0.23 (<0.1–1.09)	45.20 (<0.2–78.4)

Note: BHB negative = BHB not detected
[a] Of the total material
[b] Of the BHB-negative cases.

was AKA, accounting for 13.1% (184) of the total number of cases and 38.2% of the ketoacidotic cases. DKA was the second most common cause of death, accounting for 11.1% (156) of the total number of cases and 32.4% of the ketoacidotic cases. The combined AKA cases (cause-of-death (184) and not-cause-of-death (37), together n = 221) constituted 15.7% of the total cases. The combined DKA cases (cause-of-death (156) and not-cause-of-death (11), together n = 167) constituted 11.9% of the total cases. The cases that did not fall into any of the diagnostic categories (909) constituted 64.6% of the total number of cases (see Table 1).

In the DKA cause-of-death group, 42.3% had moderate ketoacidosis and 57.7% had severe ketoacidosis (Table 2). In the AKA cause-of-death group, 80.4% had moderate ketoacidosis and 19.6% had severe ketoacidosis (Table 3). Thus, a larger proportion of the DKA cases had severe ketoacidosis compared to moderate ($P < 0.001$).

In the female DKA cause-of-death group, 40.5% had severe ketoacidosis, while in the corresponding male group, the fraction was 63.0%. In contrast, a very large proportion of the male AKA cause-of-death cases, 82.7%, had moderate ketoacidosis. The corresponding figure for the female cases was 73.3%.

The male/female ratio was 3.2 in the DKA cause-of-death group, and 3.1 in the AKA cause-of-death group, which was not significantly different.

In the DKA cause-of-death group, the median vitreous glucose concentration was 34.2 mmol/L and the range from <0.2–78.4 mmol/L (Table 2). In the AKA cause-of-death group, the median vitreous glucose concentration was 0.6 mmol/L and the range from <0.2–17.1 mmol/L (Table 3). In the control group, the median vitreous glucose concentration was 0.8 mmol/L, and the range from <0.2–75.6 mmol/L (Tables 1–3).

In the DKA cause-of-death group, the median femoral blood alcohol concentration was 0.40‰ and the range from <0.1–1.65‰ (Table 2). In the AKA cause-of-death group, the median femoral blood alcohol concentration was 0.57‰ and the range from <0.1–3.05‰ (Table 3). In the control group, the median blood alcohol concentration was 1.18‰ and the range from <0.1–4.81‰ (Tables 2 and 3).

3.1. Age distributions based on BHB concentrations for DKA and AKA cases

Fig. 2A–C and Table 2 present the age distributions of all the DKA cause-of-death cases for the different BHB concentrations. In DKA, the cases with moderate ketoacidosis were older than the controls ($P = 0.0218$). The severe DKA cases were younger than both the controls ($P < 0.001$) and the moderate cases ($P < 0.001$). The median age for all DKA cause-of-death cases was 58 years.

Table 3
Alcoholic ketoacidosis cases, cause of death and controls.

		Number of cases	% of cases	% women (n)	Age at time of death (y), median (range)	BMI (kg/m^2), median (range)	Blood alcohol (‰), median (range)	Vitreous glucose (mmol/L), median (range)
Total	BHB negative	555	39.4%[a]	24.1% (134)	60 (18–95)	25.7 (9.9–61.9)	1.18 (<0.1–4.81)	0.8 (<0.2–75.6)
	All AKA cases	184	100%	24.5% (45)	64 (37–85)	22.2 (10.7–40.6)	0.57 (<0.1–3.05)	0.60 (<0.2–17.1)
	BHB 250–1000 µg/g	148	80.4%	22.3% (33)	65 (42–85)	22.5 (12.5–40.6)	0.59 (<0.1–2.90)	0.60 (<0.2–12.5)
	BHB > 1000 µg/g	36	19.6%	33.3% (12)	63 (37–76)	20.3 (10.7–38.5)	0.51 (<0.1–3.05)	0.51 (<0.2–17.1)
Women	BHB negative	134	24.1%[b]	100%	57 (19–90)	24.6 (9.9–56.7)	1.56 (<0.1–4.81)	0.7 (<0.2–52.8)
	All female AKA	45	100%	100%	62 (37–82)	21.8 (10.7–38.5)	0.32 (<0.1–2.90)	0.50 (<0.2–2.8)
	BHB 250–1000 µg/g	33	73.3%	100%	65 (43–82)	22.2 (14.0–35.7)	0.41 (<0.1–2.90)	0.45 (<0.2–2,5)
	BHB > 1000 µg/g	12	26.7%	100%	59 (37–76)	19.6 (10.7–38.5)	0.20 (<0.1–1.93)	0.60 (<0.2–2.8)
Men	BHB negative	421	75.9%[b]	0%	61 (18–95)	26.0 (13.6–61.9)	1.05 (<0.1–4.73)	0.8 (<0.2–75.6)
	All male AKA	139	100%	0%	65 (42–85)	22.3 (11.7–40.6)	0.61 (<0.1–3.05)	0.60 (<0.2–17.1)
	BHB 250–1000 µg/g	115	82.7%	0%	65 (42–85)	22.7 (12.5–40.6)	0.62 (<0.1–2.81)	0.65 (<0.2–12.5)
	BHB > 1000 µg/g	24	17.3%	0%	64 (47–75)	20.3 (11.7–24.6)	0.55 (<0.2–3.05)	0.45 (<0.2–17.1)

Note: BHB negative = BHB not detected
[a] Of the total material
[b] Of the BHB-negative cases.

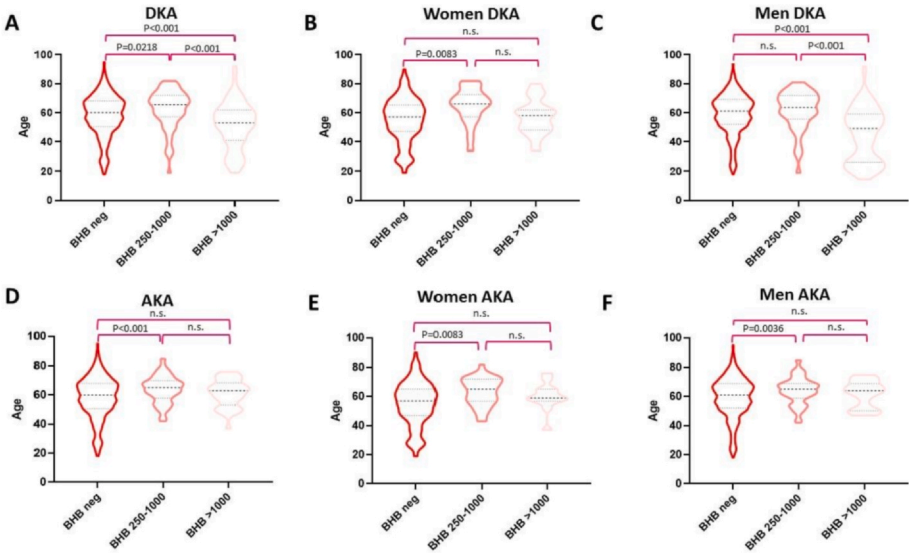

Fig. 2. Distribution of age based on BHB concentrations and sex for DKA and AKA cases. Note: n.s. - not significant, neg – negative, *i.e*, not detected, DKA – diabetic ketoacidosis, AKA – alcoholic ketoacidosis.

In the female DKA cause-of-death group (Fig. 2B), the moderate cases were older than the controls (P = 0.0083). The median age of the severe cases did not significantly differ from that of the moderate cases (Fig. 2B). In the male DKA cause-of-death group, the severe cases were younger than the controls and younger than the moderate cases (P < 0.001). The median age of the male moderate cases did not differ significantly from that of the control cases (Fig. 2C).

Fig. 2D–F and Table 3 present the results for the total AKA group. In the total AKA group, the moderate group was older than the controls (P < 0.001). The age of the severe group did not significantly differ from that of the moderate cases. The median age for all AKA deaths was 64 years (Fig. 2D and Table 3).

The results for the female AKA cause-of-death group concerning age are presented in Fig. 2E and in Table 3. In the female AKA group, the moderate cases were older than the controls (P = 0.0083). The age of the female severe cases did not significantly differ from that of the moderate cases nor of that of the controls.

Fig. 2F and Table 3 present the results for the male AKA cause-of-death group with respect to age. In the male AKA group, the moderate cases were older than the controls (P = 0.0036). The ages of the severe group and the moderate group did not significantly differ.

3.2. BMI distributions based on BHB concentrations for DKA and AKA cases

Fig. 3A–C and Table 2 present the BMI results for the DKA cause-of-death group. In this group, the severe cases had a lower BMI than both the controls and the moderate cases (P < 0.001, Fig. 3A). The BMI of the controls did not significantly differ from the BMI of the moderate cases. These differences were significant also for male cases, where the male severe cases had a lower BMI than both the male controls and the male moderate cases (P < 0.001). However, the BMI of the male moderate cases did not significantly differ from that of male controls (Fig. 3C). The BMIs for all female DKA groups did not significantly differ from each other (Fig. 3C).

Fig. 3D and Table 3 present the results for the AKA cause-of-death group. In this group, the severe cases and the moderate cases both had a lower BMI than the controls (P < 0.001). The severe cases also had a lower BMI than the moderate cases (P = 0.0391, Fig. 3A).

For the female AKA cases (Fig. 3E), the BMIs of the severe cases, of the moderate cases and of the controls did not significantly differ. In the male AKA group (Fig. 3F), the severe cases had a lower BMI than the controls and the moderate cases (P < 0.001). The male moderate group also had a lower BMI than the controls (P = 0.0469).

4. Discussion

The results of this study indicate that age and BMI differ between DKA and AKA cases with different concentrations of BHB. In DKA, the sex of the individual may also be of significance.

4.1. DKA and AKA in relation to age, BMI and sex

As far as DKA and age are concerned, severe cases were younger than the controls, and also younger than moderate cases. The same was true for male DKA cases. In contrast, moderate female cases were older than the controls, but not older than the severe cases. These results suggest that men with DKA die younger and at higher BHB concentrations, while women are older and die at lower BHB concentrations.

In AKA, moderate cases were older than the controls both in the total group, and in the male and female subgroups. No significant differences were noted between the severe cases and the moderate cases or the controls. Thus, in AKA, age does not vary with BHB concentrations to the same extent and in the same manner as it does in DKA, and does not have the same relevance.

Concerning DKA and BMI, the severe cases had a lower BMI than both the controls and the moderate cases. The same also applied to the male DKA cases. The BMI of the moderate cases did not significantly differ from that of the controls, neither in total DKA nor in its male or female subgroups. Thus, in addition to dying at a younger age, the male severe cases also had a lower BMI.

It may be that younger men neglect their diabetes to a larger extent and subsequently succumb to a more severe DKA. The generally observed link between diabetes and high BMI is also not seen in these cases. Women, on the other hand, may care better for their diabetes, and survive longer. However, when women die from DKA, they do so at a lower BHB concentration. Advanced age is a risk factor for death by DKA [25], so it is therefore unsurprising that older women die at lower BHB concentrations.

DKA in type 2 diabetes has been associated with obesity [16]. It is, therefore, surprising that the severe DKA cases had a lower BMI than the control cases and the moderate cases. Indeed, the median

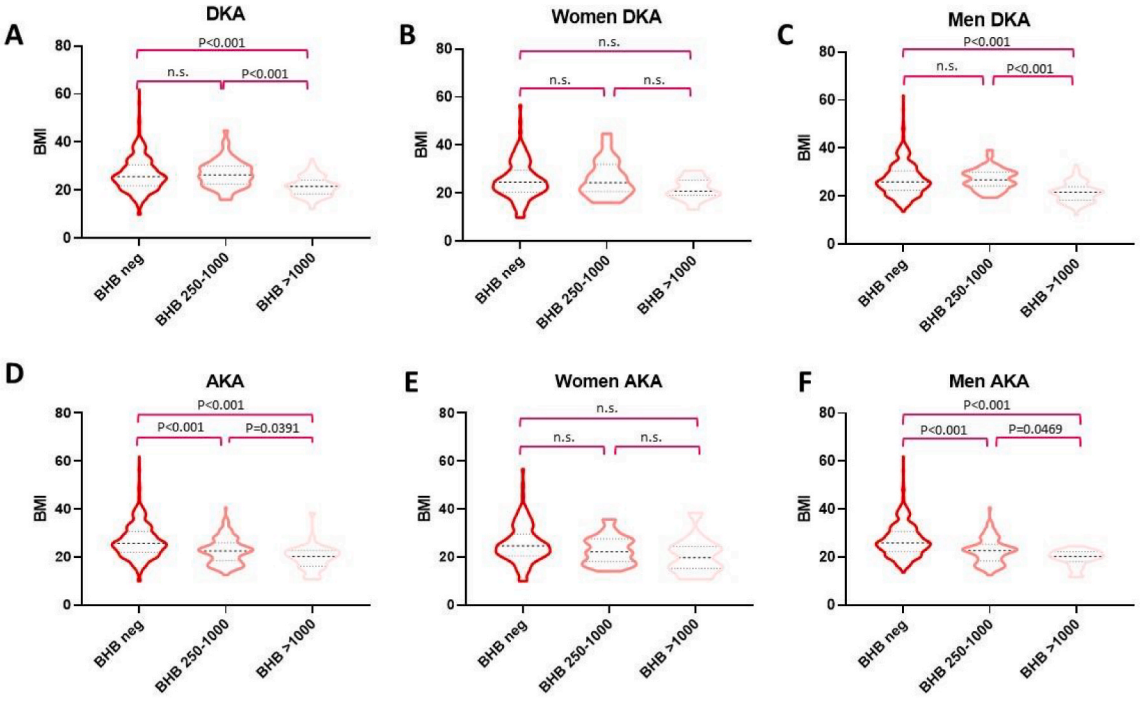

Fig. 3. Distribution of body mass index (BMI) based on BHB concentrations and sex for DKA and AKA cases. Note: n.s. - not significant, neg – negative, *i.e*, not detected, DKA – diabetic ketoacidosis, AKA – alcoholic ketoacidosis.

BMI of the severe group was in the lower normal range (21.5 kg/m^2), meaning 50% of the cases had a BMI below this value. According to the definition of the WHO, a normal BMI should be in the range 18.5–24.9 kg/m^2 [26]. Our results show that, although diabetes at diagnosis is associated with obesity, dying from a severe diabetes-induced ketoacidosis is not associated with a high BMI. In fact, the opposite is true: severely ketoacidotic individuals, who should be worse off in their diabetes, have a lower BMI than moderately ketoacidotic individuals, who presumably suffer from a diabetes that is less severe. The low BMI of the severely ketoacidotic individuals may be a consequence of them being in a ketotic state for a long period of time. A ketogenic diet, which induces ketosis, is known to cause weight loss [27].

In AKA, the importance of BMI is even more pronounced. Severe cases had a lower BMI than the controls and the moderate cases. The moderate cases, in turn, had a lower BMI than the controls. The pattern was similar for female cases, but the results were not significant. The numbers of cases were low in the female subgroups, (15 and 22), and may have been too low to reveal a small difference. Thus, in contrast to DKA, a low BMI overall was linked to AKA in chronic alcoholism and associated with its severity. Malnutrition is common in chronic alcoholism [11], and the low BMI in these cases may reflect the severity of the alcoholism and the individual's physical enfeeblement. A low BMI in a chronic alcoholic could thus raise the suspicion of possible AKA when no apparent cause of death is available and suggest a need for appropriate testing.

The results reveal only one significant association for AKA and age: moderate cases were significantly older than the controls. There are, however, some other age-related associations. The youngest victim of lethal alcoholic ketoacidosis in our material was 37 years of age at the time of death and the median age was 64 years in the total group. AKA typically develops in persons with chronic alcohol abuse [28,29]. "Chronic alcoholism" is not a well-defined term, and may not be the same as "alcohol dependency" as described in DSMV.

In conclusion, we have shown that lethal AKA generally occurs in middle-aged and older persons, when the alcohol abuse has presumably persisted for a number of years, and when alcohol-related physical signs are evident and general physical decline might be more pronounced.

Type 2 diabetes is somewhat more prevalent among men than women in Sweden, and men develop diabetes at a younger age and at a lower BMI than women do [30]. The male/female ratio was 1.3 in one study, while it was 1.1 in another [31]. The male/female ratio of the DKA cases in our material was much higher: 3.2.

The proportion of women was nearly the same in the DKA and AKA categories, 23.7% and 24.5%, respectively, which was approximately the proportion in the total material, 25.0%. The figures are also approximately the same as the overall proportion of women who undergo a forensic autopsy in Sweden, which in 2018 was about 26.6%. The proportion of men who undergo a forensic autopsy is always higher than that of women, since more men than women die in circumstances that by law require a medico-legal investigation [32].

4.2. DKA and AKA in relation to vitreous glucose and blood-alcohol

Our findings on BHB and vitreous glucose concentrations in DKA and AKA agree with previous results: the BHB values and vitreous glucose concentrations in DKA are usually higher than in AKA. However, there is some overlap between the glucose concentrations in DKA and AKA, even in the clinical setting [33]. Several DKA cases also had low vitreous glucose concentrations, and some were even below the detection threshold (<0.2 mmol/L). Thus, it is not possible to differentiate between DKA and AKA based on vitreous glucose concentrations alone. Much research has been concentrated in recent years on the interpretation of *post-mortem* vitreous glucose concentrations. This parameter is the most reliable marker for hyperglycaemia [21,34,35]. However, as vitreous glucose concentrations still are unstable *post mortem*, DKA can exist simultaneously with a low vitreous glucose concentration and an elevated

ketone body concentration is the primary diagnostic criterion for DKA [19]. BHB, unlike vitreous glucose, is stable *post mortem* [3,36,37]. Thus, differences in *post-mortem* intervals might have affected the decisions in some of the cases, but the high number of cases in this study has hopefully minimized this influence. Based on our results, one should not stare blindly at vitreous glucose concentrations when determining the cause of a ketoacidotic state, but consider all available information.

Blood-alcohol concentrations tend to be low or even absent in AKA, and the condition typically evolves after a short period of abstinence [3,4,28,36,38]. There is an inverse relationship between blood-alcohol and ketone body concentrations [24,39,40]. Our results confirm that alcohol concentrations are generally low in AKA, although a few cases have high alcohol, the highest measuring 3.05‰. A high alcohol concentration therefore does not rule out ketoacidosis and the need for BHB analysis should not be excluded based on a high alcohol concentration.

The comparatively high median alcohol concentration of the control group, 1.18‰, is a consequence of the fact that substance abuse, including alcoholism, is one of the criteria for a medico-legal investigation in Sweden. High alcohol concentrations are frequently encountered at autopsy.

4.3. Hyperosmolar hyperglycaemic state and hypothermia

HHS was considered to be cause of death in only eight cases of the 1407 examined, and it was the cause of death in all cases in which it was present. In contrast, DKA was considered cause of death in 156 cases, while the condition was encountered in a further 11 cases where it was not cause of death. Thus, HHS is not a frequent cause of death. However, one must keep in mind that BHB was analysed in all the cases presented here. HHS may have been established in other cases during this period based on glucose values alone. Further, cases of HHS in which the glucose concentration was low or undetected due to *post-mortem* changes may have been missed.

It is also worth noting that in the category “other causes of death”, some very high vitreous glucose values were noted. These were classified as “others”, because BHB was not elevated, the cause of death was unrelated to diabetes, and often suicidal or accidental (for example suicidal intoxication, traumatic cervical fracture, suicidal slashing of the wrist, and hanging).

Thirty-seven cases were encountered in which the BHB concentration was above 250 µg/g and the elevation was alcohol-related, but AKA was not considered cause of death. In contrast, only 11 cases of DKA had other causes of death than DKA. Thus, ketoacidosis is more lethal for diabetics who die with an acidosis than it is for chronic alcoholics who die with ketoacidosis.

The median BHB concentration was 390 µg/g in those for whom hypothermia was cause of death, and the range was from 81 to >1000 µg/g. Of these cases, 72.4% hadmoderately elevated BHB concentration between 250 and 1000 µg/g. These findings agree with previous results on hypothermia. Palmiere et al. reported a median blood BHB concentration of 1850 µmol/l (182 µg/g) in a study of ten fatal hypothermia cases. The range was 300–6700 µmol/l (30–658 µg/g) [41]. Teresinski et al. studied 16 hypothermia cases, and found that blood BHB lay in the range 274–23855 µmol/l (27–2343 µg/g) [24].

4.4. Limitations

DKA and AKA lack a clear scientific definition, and inclusion of cases into these causes of deaths may have been biased. These causes of death require background information on alcoholism and diabetes, which was sometimes missing, either because medical records had not been requested or because the deceased had not been in contact with the healthcare system. Thus, for example, some cases with high vitreous glucose concentrations and known alcoholism did not have information on diabetes, and the cause of death in these cases was considered to be AKA. Had these persons been in contact with the healthcare system while alive, they would probably have been diagnosed with diabetes.

5. Conclusions

In conclusion, the study results indicate that age and BMI differ between different concentrations of BHB in DKA and AKA, and that the correlations are also affected by the sex of the individual. Severe DKA cases in men are associated with a lower age and a lower BMI, but for women the effects are less pronounced. For AKA cases, the age is of less significance. In AKA, a lower BMI can be seen with increasing BHB concentrations in the total group and with men. These results show that in a *post-mortem* setting, age, BMI and sex have an impact on the ketoacidosis and should be considered when interpreting BHB concentrations. A low BMI in a known alcoholic with no apparent cause of death should therefore raise the suspicion of a possible AKA. A low BMI cannot be used as indicator against DKA, even though obesity is associated with diabetes.

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Ethical approval

Approval was obtained by the Regional Ethics Committee in Linköping (Dnr 2016/489–31). The procedures used in this study adhere to the tenets of the Declaration of Helsinki.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Authors’ contribution

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by SA, AKJ and HG. The first draft of the manuscript was written by SA and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

CRediT authorship contribution statement

Stina Ahlström: Conceptualisation, Methodology, Formal analysis, Investigation, Writing - original draft, Writing - review & editing. **Ingemar Thiblin:** Conceptualisation, Formal analysis, Investigation, Writing - review & editing. **Anna Jönsson:** Methodology, Formal analysis, Writing - review & editing. **Henrik Green:** Conceptualisation, Methodology, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.forsciint.2021.110878.

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