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PAPER

PATHOLOGY/BIOLOGY

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Postmortem Vitreous Beta-Hydroxybutyrate: Interpretation in a Forensic Setting*

ABSTRACT: Vitreous beta-hydroxybutyrate (BHB) was retrospectively analyzed in 1795 forensic cases using the Pointe Scientific method. Comparison of vitreous BHB with vitreous glucose in 1781 of the cases showed moderately good correlation $r = 0.731$. Comparison with blood alcohol levels in 1561 of the cases showed no correlation $r = -0.053$. Vitreous BHB was a marker of diabetic ketoacidosis when above 6.0 mM with a vitreous glucose over 200 mg/dL. It was an indicator (>50%) for alcoholic ketoacidosis when above 6.0 mM with a vitreous glucose below 200 mg/dL. Recommendations for interpretation of vitreous BHB: <0.4 mM normal; 0.41–1.2 mM slightly elevated, rarely (<1%) of concern; 1.21–2.0 mM moderately elevated, less rarely (2.5%) of concern; 2.01–6.0 mM significantly elevated, frequently of concern (12–48%); >6.0 mM usually (100% in this study) indicated life-threatening conditions. Vitreous BHB was helpful evaluating cases with ketogenic conditions, especially diabetes and alcoholism.

KEYWORDS: forensic science, postmortem chemistry, death investigation, beta-hydroxybutyrate, ketones, vitreous fluid, diabetes, alcoholism

New onset of insulin-dependent diabetes mellitus often presents with ketoacidosis that can be rapidly fatal. Ketones are used to determine when uncontrolled diabetes is leading to life-threatening acid–base disturbances. Three substances have been used to measure ketoacidosis: acetone, acetoacetate, and beta-hydroxybutyrate (BHB). During ketoacidosis, BHB is found in the highest concentrations (1).

Blood and urine are the preferred samples to test for ketones in clinical practice. Blood, vitreous fluid, and other body fluids have been utilized in postmortem investigation. Vitreous BHB is an attractive alternative when blood BHB is not used in postmortem analysis (2). Blood BHB and vitreous BHB show good correlation (3–5).

The lack of insulin function in diabetes does not allow glucose to be used as an energy source (1). Fatty acids become the primary source of energy in the setting of decreased utilization of glucose, the usual primary metabolic fuel. BHB and the other ketones are metabolites of free fatty acids. When the liver's capacity to metabolize the final fatty acid catabolite (acetyl COA) is exceeded, ketones accumulate and acidify the blood (2). Ketones can be used by the brain as an energy source when glucose is not available (6).

Blood glucose levels usually fall to zero within a few hours after death. Postmortem blood glucose levels can also be falsely elevated. Postmortem glycogenolysis in the liver increases glucose diffusion into the vena cava and right ventricle of the heart. Terminal stress with catecholamine release and intravenous fluids containing glucose administered during resuscitation also elevate postmortem blood glucose in sites away from the liver. Hence, blood glucose levels are not reliable in the evaluation of diabetes during the postmortem period (7). However, glucose levels measured in vitreous fluid have been shown to never exceed 200 mg/dL except in the setting of diabetes (7). Vitreous glucose levels also decrease after death. But only rarely does this postmortem decrease entirely eliminate the elevated vitreous glucose in the poorly controlled diabetic. Thus, diabetic ketoacidosis (DKA) can be reliably diagnosed in the early postmortem period by an elevated vitreous glucose (greater than 200 mg/dL) and elevated ketones (7).

Elevated BHB is also a feature of alcoholic ketoacidosis (3). Ethanol is metabolized to acetaldehyde and then to acetate. Both of these steps generate nicotinamide adenine dinucleotide hydrate (NADH). Increased NADH down-regulates the citric acid cycle and decreases gluconeogenesis. When an alcoholic does not ingest adequate carbohydrates, glycogen reserves are depleted with further reliance on lipids. Other starvation states and severe infectious or inflammatory diseases are associated with increased levels of ketones and normal glucose levels. When no obvious anatomic cause of death (except perhaps a fatty liver) is found at the autopsy of an alcoholic along with ketones being elevated, the diagnosis of alcoholic ketoacidosis can be considered (8).

This retrospective study was performed to better understand the frequency and usefulness of postmortem vitreous BHB levels in our medical examiner setting. The conditions associated with increased BHB are common. The medical examiner is often faced with an elevated vitreous BHB that appears to have little or no bearing on the case.

Hypothesis

Postmortem vitreous BHB is a useful marker for DKA when levels are elevated above 6.0 mM and accompanied by vitreous glucose levels above 200 mg/dL. Postmortem vitreous BHB often indicates DKA when in the 2–6 mM range and accompanied by similarly elevated glucose levels. Postmortem vitreous BHB often

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indicates alcoholic ketoacidosis when levels are elevated above 6.0 mM and accompanied by vitreous glucose levels below 200 mg/dL.

Methods

The vitreous fluid samples were collected from genuine medical examiner cases during autopsy or external examination. All cases (15,837) reported to the Fulton County Medical Examiner from 2003 to 2009 were electronically searched retrospectively for cases where BHB was measured in vitreous fluid. BHB was not measured in most cases that came for examination, but only in cases when the physician thought it might prove useful, for example, evaluating the control or presence of diabetes. Vitreous BHB was more likely to be measured in natural than violent deaths (25% vs. 10% or less).

Vitreous BHB was effectively the only test available at the time of this study to evaluate ketoacidosis. Most samples were collected in the morning, and no unusual effort was made to keep the postmortem interval especially short. The samples were collected by a physician or a forensic technician inserting a syringe into the globe of the eye through the sclera and drawing out vitreous fluid. It was then placed into a test tube (red top) without any other substances or preservatives. Fluid from both eyes was usually placed into one tube. Severely putrefied cases excluded themselves when vitreous fluid was no longer easily obtained or when the clinical laboratory refused to test for BHB. The samples were refrigerated after the procedure (usually within 1 or 2 h). The transportation to the laboratory was usually carried out the same day, and the distance was only 1 to 2 miles. BHB was one of the tests performed as part of the vitreous electrolyte analysis. The other ketones were not measured by the laboratory and not included in the study, although acetone occasionally appeared during testing for blood alcohol at another laboratory, available many weeks later.

Analysis of vitreous BHB was performed using a standard clinical laboratory method. Vitreous BHB was measured for the entire 6 years of the study by the Pointe Scientific method developed for serum. Enzymatic quantization of D-3-hydroxybutyrate was measured with BHB dehydrogenase in the presence of NAD+ at a pH of 8.5 resulting in the production of acetoacetate and NADH. The NADH product was converted to color using iodonitrotetrazolium violet dye (INT) with diaphorase and read at 505 nm. Pointe Scientific reagent package insert and other information is available online at http://www.pointescientific.com (9).

Results

BHB was measured in vitreous fluid in 1795 cases. Laboratory and demographic data, cause and manner of death, and glucose and alcohol levels of these 1795 cases were assembled into a data file. The 1795 vitreous BHB levels in this exceedingly variable population did not follow a statistically normal distribution ranging from 0 to 22.7 mM with an average of 1.207 mM, a median of 0.81 mM, and a standard deviation of 2.08 mM (Table 1). The variation of average vitreous BHB levels from year to year (Table 1) was statistically significant and not because of a random distribution (Kruskal–Wallis test, $H = 59$, $p < 0.0001$).

The median age was 53 years, ranging from 14 h to 97 years. The gender ratio was 65.5% male and 34.5% female. The racial and ethnic distribution was 67.1% black, 30.2% white, and 2.67% every other designation including Hispanic, black or white Hispanic, Asian, and unspecified. The proportion of both gender and race was close to 50% in the general population of the county served. The data were skewed in the direction of higher numbers of cases of both males and blacks, and even more so at the higher levels of BHB. The average vitreous BHB for males was 1.18 mM and for females it was 1.26 mM. The average vitreous BHB for blacks was 1.21 mM, and for whites, it was 1.18 mM. Neither of these differences of average BHB levels between sex and race was statistically significant (two-tailed heteroskedastic Student's *t*-test of 0.44 and 0.77, respectively).

TABLE 1—*Number of cases and descriptive statistics for each year.*

Year	Number of Cases	BHB Average (mM)	BHB Median (mM)	Standard Deviation (mM)
2003	257	1.24	0.6	2.08
2004	196	1.86	1.1	3.25
2005	134	1.18	0.89	2.09
2006	214	1.22	0.73	2.08
2007	257	0.87	0.58	1.47
2008	333	0.98	0.78	1.18
2009	404	1.27	0.85	1.97
Total	1795	1.207	0.81	2.08

BHB, beta-hydroxybutyrate.

This database file of 1795 cases was sorted into five interval groups based on the level of measured vitreous BHB resulting in the frequency distribution illustrated in Table 2. The first group (A) was the given normal range for BHB (<0.4 mM), and the second group (B) was slightly elevated but below the average (0.4–1.2 mM). The 2.0 and 6.0 mM levels dividing the three groups with higher levels (C, D, and E) were arbitrary.

Glucose

Vitreous glucose levels were measured in 1781 of the 1795 cases (99.2%). In the lowest three BHB interval Groups A through C (BHB < 2 mM), the average glucose levels were low (25–35 mg/dL). Some of the glucose levels were higher in BHB Group D (BHB of 2–6 mM) increasing the average glucose to 131 mg/dL and many of them had DKA. In Group E with BHB above 6.0 mM, the average vitreous glucose was 604 mg/dL. The standard deviation also increased from 39–61 mg/dL in the first three groups to 208–286 mg/dL in the last two groups, reflecting greater variability in vitreous glucose levels in the groups with higher BHB. The increase in levels of BHB in each of the glucose groups defined above was extremely statistically significant (Kruskal–Wallis test, $H = 196$ and $p \leq 0.0001$).

No case with a glucose level over 400 mg/dL was observed in the samples with BHB less than 1.2 mM, and no cases of hyperglycemic hyperosmolar state (HHS) appeared in the study. The Pearson correlation coefficient for vitreous BHB and vitreous glucose was 0.731. Even though glucose levels decrease following death, a moderately good correlation remained between vitreous BHB and vitreous glucose.

TABLE 2—*Frequency distribution of five interval groups of vitreous beta-hydroxybutyrate levels.*

BHB Level	mM	Number of Cases	%
Group A	<0.4	562	31.3
Group B	0.41–1.2	637	35.5
Group C	1.21–2.0	439	24.5
Group D	2.01–6.0	105	5.85
Group E	>6.0	52	2.9
Total		1795	100

Alcohol

Vitreous alcohol levels were available in only 18.8% of cases (337 of the 1795). Blood alcohol levels were available in the electronic database for 1561 of the 1795 cases with BHB measured in the vitreous fluid (87%).

At every level of alcohol, 70–85% of the cases had low BHB levels (Group A or B). Only four cases showed both a vitreous BHB level above 2.0 mM and a blood alcohol level above 200 mg/dL. The Pearson correlation coefficient for vitreous BHB and blood alcohol was −0.053, indicating very little correlation.

Analysis of Each Group

Vitreous levels of BHB in Groups A and B (<1.2 mM) did not support a diagnosis of DKA without other much more compelling evidence. Vitreous glucose was rarely elevated beyond 200 mg/dL, almost always in cases of known diabetes, and never above 400 mg/dL. Apparently, if enough insulin was present to keep the BHB this low, then it was enough to keep the glucose from extremely high levels.

Vitreous BHB levels in Group C were moderately elevated above average (1.2–2.0 mM). The evaluation of BHB was less straightforward in this group. It was up to individual medical examiners to determine the relative weight placed on the BHB level and the presence or absence of ketogenic conditions. BHB in this range along with an elevated glucose supported a diagnosis of DKA, but other conditions were often convincing as explanations for the death.

Medical history and the database were carefully evaluated for all 105 cases in Group D, the group with the second to highest BHB level (2–6 mM). DKA, defined as an elevated BHB (>2 mM) and a vitreous glucose of >200 mg/dL, was observed in 24 of these 105 cases (22.9%). Group D cases included vitreous glucose levels elevated beyond 400 mg/dL in 16 of 105 cases (15.2%). Of the 81 cases with a glucose of <200 mg/dL in Group D, 19 of them (23.5%) had a history of diabetes bringing the total number of cases of diabetes in Group D to 43. Only three cases occurred in the top portion of Group D, with a BHB in the 5–6 mM range. One had a history of diabetes, and all three had vitreous glucoses less than 50 mg/dL in contrast to what was observed in Group E.

The proportion of cases with elevated glucose levels and DKA was lower in Group D when the BHB was below 3.0 mM and resembled Group C. In the 73 cases with lower BHB (2.0–3.0 mM), only nine cases (12.3%) had glucose levels above 200 mg/dL (DKA), another 12 cases (16.4%) had glucose levels in the 50–200 mg/dL range, and the remaining 52 cases (71.2%) had glucose levels below 50 mg/dL. In the 32 cases with higher BHB levels (3.0–6.0 mM), 15 of them (46.9%) had glucose levels above 200 mg/dL (DKA), and the other 17 (53.1%) had glucose levels below 50 mg/dL. The frequency of DKA exceeded 50% at BHB levels above 3 mM.

Of the 105 cases in Group D, 21 cases (20%) had a history of alcoholism or were intoxicated (>100 mg/dL) at the time of death. Frequently, we described this as complications of chronic alcoholism instead of alcoholic ketoacidosis. Not one of these 21 alcoholics had a glucose level greater than 200 mg/dL, and the highest glucose level among them was only 60 mg/dL. Both a history of alcoholism and diabetes with low glucose were noted in five of these cases. One disturbing case of a 3-year old reportedly given too much vodka died of an acute cardiac dysrhythmia with a right coronary artery anomaly, and he had a BHB level of 2.2 mM. No alcohol remained in his system but he could have been considered the 22nd alcohol-related case in this group. The elevated BHB level in this 3-year old was evidence of either excessive alcohol consumption and/or some element of nutritional neglect.

Medical history and the database were carefully evaluated for all 52 cases in Group E (BHB > 6.0 mM). Group E showed vitreous glucose elevated beyond 400 mg/dL in 44 of 51 cases (86.3%), and these were obvious cases of DKA. Among the remaining eight cases in this group, four had a history of diabetes bringing the total of diabetics to 48. Alcoholism was noted in four of the eight cases when BHB was in this elevated range and glucose levels were low. Alcoholic ketoacidosis was observed in three cases (7.5%), defined as elevated vitreous BHB, no history of diabetes, glucose lower than 200 mg/dL and a history of chronic alcoholism. Another case had a blood alcohol level of 237 mg/dL and a history of only atherosclerotic heart disease, another likely alcoholic.

Four of these 44 indisputable cases of DKA also had a history of chronic alcoholism. Included in these 44 DKA cases were another three with pancreatitis and one with an upper gastrointestinal bleed. Although alcohol use was not recorded in the medical history, it probably played a role in these four cases. The number of alcohol-related deaths in this group of 44 DKA cases was at least eight to possibly 11. Either diabetes or alcoholism was found in every one of these 52 cases with markedly elevated BHB. No case with a serious infection was noted at this level of BHB; three cases had pancreatitis, a serious inflammatory condition usually related to alcoholism.

Not all cases in Group E had natural manners of death. Violent manners of death included one suicide by carbon monoxide poisoning simultaneous with DKA, another accidental food poisoning that led to DKA, and a third accidental water immersion with chronic ischemic heart disease and diabetes.

Analysis without BHB Levels

The usefulness of BHB was demonstrated in some cases where it was not obtained. The same database that produced the 1795 BHB cases also included 2121 cases where vitreous glucose was measured including 326 additional cases with vitreous glucose available but no accompanying BHB level. Most (305 or 93.6%) of these 326 cases showed low vitreous glucose levels. An elevated BHB level might have supported a designation of alcoholic ketoacidosis in this large group. Alcoholism was in the history of 27 of them (8.9%), and two of these were diagnosed as alcoholic ketoacidosis without a BHB level, based on elevated acetone levels in the toxicology screening for alcohol.

In 21 (6.4%) of these 326 cases, the vitreous glucose was elevated above 200 mg/dL and 12 had DKA. This was based on acetone detected in the screening for alcohol coupled with an elevated glucose, consistently above 400 mg/dL in each of these 12 cases. The other nine of these cases with elevated glucose either had a history of diabetes or in one case diabetes was diagnosed based on the vitreous glucose of 367 mg/dL. All nine of these cases might have been more specifically classified as either DKA or HHS depending on a BHB level. Two of these 12 DKA cases also had a history of alcoholism. Serious infections accompanied two of the 12 cases of DKA and one of the other nine cases.

One case had a vitreous glucose of 590 mg/dL and no acetone. A second similar case had a vitreous glucose of 502 mg/dL and acetone "present but below the lowest calibrator." A BHB level was not measured in either one where it would have been useful to distinguish DKA from HHS.

TABLE 3—*BHB levels and number of cases with ketogenic conditions.*

BHB Level	mM	Total Cases	Diabetes	Alcoholism	Severe Infections	No Ketogenic Conditions
Group A	< 0.4	562	91 (16.2%)	69 (12.3%)	41 (7.3%)	371 (66.0%)
Group B	0.4–1.2	637	91 (14.3%)	71 (11.1%)	58 (9.11%)	427 (67.0%)
Group C	1.21–2.0	439	65 (14.8%)	34 (7.7%)	52 (11.8%)	301 (68.6%)
Group D	2.1–6.0	105	43 (41.0%)	21 (20.0%)	19 (18.1%)	31 (29.5%)
Group E	>6.0	52	48 (92.3%)	8 (15.4%)	0	0
Total		1795	338 (18.8%)	203 (11.3%)	170 (9.47%)	1130 (63.0%)

BHB, beta-hydroxybutyrate.

Analysis by Ketogenic Conditions

Although it was not possible to obtain and review the medical history of all 1795 cases, available data from death certificates and medical history were used to compare the burden of common ketogenic conditions (diabetes, alcoholism, severe infection or inflammatory condition, starvation) in Groups A through E. The number of cases with a ketogenic condition was tallied by BHB group as indicated in Table 3.

Diabetes and alcoholism was likely underreported both on the death certificates and in the medical history. Therefore, the number of cases with no ketogenic conditions (1130) was probably overestimated. Many cases had multiple ketogenic conditions listed on the death certificate. Hence, the sum of the numbers of diabetes (338), alcoholics (203), seriously infected (170), and nonketogenic conditions (1130) in Table 3 exceeds the total number of cases (1795). In this study, cases of starvation, malnutrition, or neglect were encountered only eight times and a separate column was not included in Table 3.

Nearly 19% of all 1795 cases had diabetes, and this portion was only slightly underrepresented in the first three groups (14–16%). Most (247 of 338 or 73.1%) of the time diabetes was encountered the BHB level was below 2.0 mM. The portion with some form of diabetes on the death certificate increased to 41% and 92.3% in Groups D and E with increasing levels of BHB, but they made up only 26.9% of the diabetics.

The average BHB level in the 338 known diabetics was 2.63 mM compared with the average BHB in the rest of the cases of 0.877 mM, and this was extremely statistically significant. (Mann–Whitney test, $Z = 6.02$, $p < 0.0001$). The average BHB level in the 1130 cases with no identified ketogenic conditions was 0.820 mM compared with the other 665 cases with one or more ketogenic conditions where it was 1.87 mM. This was extremely statistically significant (Mann–Whitney test, $Z = 5.34$, $p < 0.0001$).

The average BHB level of 203 cases of alcoholism was 1.33 mM, and this was not statistically significantly different from the BHB average of all cases (1.207). The variation of the average BHB level from year to year (Table 1) ranged from 1.86 to 0.87 mM, and this was similar to the differences between the statistically significant averages above.

Discussion

Interpretation

In these 1795 cases, 31.3% of the measured vitreous BHB levels were <0.4 mM, the cited normal range for BHB. Cases with BHB > 2.0 mM amounted to only 8.8%. This left around 60% of the values in the range of 0.4–2.0 mM (Group Bs and C in Table 2). These slightly elevated levels were more difficult to evaluate. Other factors were needed to support a determination of DKA as a cause of death at BHB levels <2 mM.

In the rare instance when an elevated vitreous glucose was encountered with a BHB level in the 1.2–2.0 mM range (11 out of 439 cases), a history of diabetes was usually found (10 of the 11 cases). A history of ketogenic conditions was not uncommon at lower levels of BHB, collectively amounting to around 1/3 of the cases. The BHB level did not usually play a role in determining the cause and manner of death when it was only modestly elevated in the 0.4–2.0 mM range.

Interpretation was not difficult in cases where both BHB and glucose were markedly elevated (BHB > 6 mM and glucose > 400 mg/dL) or where both were low (BHB <0.4 mM and glucose <50 mg/dL). DKA was demonstrated in the former situation and no indication of anything abnormal was suggested in the later. HHS would be likely when glucose levels were markedly elevated and BHB was normal, but was not encountered in this study.

Most of the 51 glucose levels in Group E (BHB > 6 mM) were markedly elevated with an average of over 600 mg/dL. But in six cases in this group, the glucose level was under 200 mg/dL. Three of these six cases (50%) had a history of alcoholism and a fourth had a blood alcohol level of 237 mg/dL. In the uncommon situation of extremely high ketones and low glucose, consideration of an alcohol-related cause of death was warranted. In Group D with BHB in the 2–6 mM range, this observation did not hold up quite as well. Alcoholism was observed in the history of 21 of the 81 cases (25.9%) in Group D with a glucose level below 200 mg/dL and was still worth considering. Either diabetes or alcoholism or both was a significant factor in the death of every case with a BHB > 6.0 mM. The trend lessened below that level. In the range of 2–6 mM, one or more ketogenic conditions were found in about 70% of the cases.

Limitations

This study was a retrospective analysis and was not initially designed to answer any specific questions. A number of problems with the data limited its usefulness. Selection bias was evident when only 11.5% of the total cases presenting for evaluation in that period had BHB measured. The selection of these 11.5% of cases was not arbitrary but highly biased in the direction where BHB was thought to be useful by the medical examiner. Indication bias was evident when BHB was ordered more often in cases where it was more likely to be elevated. For example, only 2.27% of homicides were tested in BHB, while 26.1% of natural deaths were tested for BHB.

Confirmation bias was evident when elevated vitreous BHB was used as a major criterion for the diagnosis of DKA. Other independent markers for DKA were effectively not available in the day-to-day practice from which this data were derived. Therefore, this study did not independently demonstrate a specific level of vitreous BHB that was significant. Rather this study described the level of BHB that a group of forensic pathologists who work closely together (and most were trained at the same institution) more often

than not made a diagnosis. Consideration of other factors was entertained but the BHB level was crucial, not independent in the determination of DKA and also in a few other cases of diabetes that were diagnosed postmortem.

The provided normal level was determined from premortem blood BHB levels and it has changed. For most of the study, it was at 0.4 mM but recently it has been lowered to 0.27 mM with no change in the method used to test for vitreous BHB. Pounder et al. showed that generally femoral vein BHB tended to be nearly twice as high as vitreous BHB (4). Felby et al. also showed that blood BHB was generally higher than vitreous BHB (3). In light of this, most of our vitreous BHB levels were probably higher than their corresponding blood BHB levels.

Comparison with Other Studies

Human populations encountered in these comparisons were not consistent from place to place. Selection criteria that often reflected the level of resources available and the priorities and bias of the researcher were frequently different. In a few studies, they were not even defined. Some studies focused on diabetes, others on alcoholism and a few on both.

Osuna et al. (2) published a study in 2005 involving 453 deaths, divided into a group of 111 cases with the antemortem diagnosis of diabetes, based on medical records that were compared with a group of 342 cases without such a diagnosis. Vitreous humor showed statistically significant differences between the two diagnostic groups in five biochemical markers including BHB, glucose, fructosamine, lactate, and the sum of glucose and lactate (2). This was similar to our finding for vitreous BHB in cases with and without diabetes.

Felby et al. (3) compared BHB, acetoacetate, and acetone levels in 105 forensic autopsies in blood, spinal fluid, vitreous fluid, and urine. Spinal fluid showed the best correlation with blood followed by vitreous fluid, and these correlations were described with regression lines. The vitreous fluid BHB levels tended to be lower than the blood BHB levels, attributed to protein binding in blood, and differences in dry weight of vitreous fluid and blood (3).

Pounder et al. (4) looked at all three ketones collected from up to six sites (vitreous, pericardial, femoral vein, inferior vena cava, superior vena cava, and aorta). A total 105 forensic autopsies carried out in Scotland and reported in 1998 included 22 alcoholics, 12 diabetics, and 71 controls. Total ketones in vitreous fluid showed good correlation with blood and pericardial fluid. The ratio of femoral vein ketones to vitreous fluid ketones was almost 2:1. Pounder et al. (4) found that 10% of their chronic alcoholics who came to forensic autopsy had elevated ketones, >5 mM in vitreous fluid, and >10 mM in blood. In our group of 203 alcoholics, only 9 (4.4%) had a vitreous BHB > 5 mM.

Gagajewski et al. (5) published a study in 2004 that included measurement of BHB in 24 medical examiner autopsies among a wider comparison of several chemical constituents in vitreous fluid from the right and left eyes in 126 total forensic autopsies. They looked at frozen and refrigerated samples for 6–12 months and confirmed stability and reliability. Their study showed that vitreous BHB was a good reflection of blood BHB (5).

The average vitreous BHB level in these 48 samples from 24 individuals (selected for possible ketoacidosis) was 1.98 mM, which was higher than our 1.207 mM average. Twenty of the 24 (83%) were considered to be elevated, being >0.4 mM. In our study, 69.3% were above this level. Diabetes was listed as the cause of death in five of these cases (21%) and alcoholism was listed in three of them (2.5%). This corresponded relatively well with our rate of diabetes at 18.8% and alcoholism at 11.5%. No acetone was detected in any of their samples, and only a trace of acetoacetate was in one sample. Vitreous BHB was a considerably more sensitive determinant of ketosis than acetone or acetoacetate (5).

Elliot et al. (10) measured ethanol, acetone, and BHB in the blood, urine, and vitreous fluid of 350 fatalities grouped into alcoholics, diabetes, both, and other causes of death. They found that ethanol was not always low in alcoholic ketoacidosis. They also found cases with elevated BHB levels, and no acetone was detected. They suggested an interpretative range for blood and urine BHB that could also be applied to vitreous fluid: normal (<0.48 mM), raised (0.49–2.49 mM), high and pathologically significant (>2.50 mM) (10). If we applied the interpretative ranges of Elliot et al. to our 1796 cases, we would have 633 (35%) normal, 1058 (59%) raised, and 104 (6%) high and pathologically significant. All of our Group E and half of the highest 52 of our Group D would fall in this high range. This fits remarkably well with what we observed.

Iten and Meier (11) suggested that blood BHB was an indicator of alcoholic ketoacidosis in chronic nondiabetic alcoholics who died suddenly with negative autopsy findings. They looked at the blood BHB in 25 chronic alcoholics who died suddenly with negative autopsies including histology, microbiology and toxicology studies, and including low or zero ethanol levels. Controls included 69 cases with blood BHB below 0.5 mM. Blood BHB ranged in the alcoholics from 1.26 to 47.2 mM with a median of 8 mM (11). This fits with our observation in principle that when a high BHB level was observed with a low glucose level alcoholism was often present.

Iten and Meier determined in their setting that a level of less than 0.5 mM could be considered normal, a level up to 2.5 mM could be considered elevated and levels above this could lead to death (11). This was close to the levels given by Elliot et al. (10) and Thomsen et al. (12). If the interpretations of Iten and Meier (11) were applied to our study of 1796 cases, then we would have similar results with 641 (35.7%) normal levels, 1055 (58.7%) elevated level, and 99 (5.5%) potentially deadly levels. The potentially deadly levels would include all of Group E and the highest 47 of 105 of group D.

Thomsen et al. (12) measured postmortem blood BHB in 131 deaths in Denmark including 79 nonalcoholics, 35 alcoholics with obvious causes of death, and 17 alcoholics without ascertainable causes of death. The geometric mean levels of BHB were 0.109, 0.152, and 0.59 mM. In alcoholics without any ascertainable cause of death including diabetes and blood BHB > 0.53 mM, the term "ketoalcoholic death" was suggested (12, p. 163). The geometric mean vitreous BHB for our 1130 cases with no ketogenic condition was 0.479 mM, which was four times higher than the blood BHB (0.109 mM) in these Danish nonalcoholics. The geometric mean BHB of our 195 alcoholics without diabetes was 0.450 mM, which was three times higher than the Danish group of alcoholics with an obvious cause of death (0.152 mM). The geometric mean BHB of our 338 cases with diabetes was also higher at 0.867 mM.

Denmark (8) studied 49 cases from England and Canada that included vitreous and urine BHB. He suggested that BHB could be used as a positive marker for hypoglycemia in the sudden deaths of chronic alcoholics with a negative autopsy except a fatty liver. His frequency distribution of BHB was different: 28 cases (60%) were below 0.4 mM and eight cases (17%) between 0.4 and 1.2 mM. He had two, six, and three cases with BHB levels that would sort into our Groups C, D, and E, respectively. Denmark's cases included 13 chronic alcoholics, nine more with substances including alcohol, and two of his natural disease cases had alcoholic liver disease for a total of 24 (51%) alcoholics in his study

(8). Our study only had 11% alcoholics. The cases with the higher levels of BHB were often alcohol related (seven of nine) in Denmark's study. In our study, the portion of alcoholics did not increase as much with increasing BHB levels. Denmark's (8) study included only three diabetics (6.4%), and all of them had BHB levels less than the average of 1.2 mM. Diabetes was present in 18.8% of our cases with an average BHB of 2.63 mM. No cases of DKA were included in Denmark's study. His study included only four cases of serious infections, and the BHB in three of the four was <0.4 mM. The average vitreous BHB level for Denmark's 47 cases in which it was measured was 1.23 mM, and the standard deviation was 2.16 mM (8). This was remarkably close to our average of 1.207 mM and standard deviation of 2.08 mM.

The study by Denmark included three cases of hypothermia all related to alcoholism and two had elevated BHB levels (8). In our study, 10 cases of hypothermia had an average BHB of 0.95 mM and a range of 0.06–1.58 mM, which was lower than the average for the rest of the cases (1.2 mM). The glucose levels in our 10 hypothermia deaths were low with a range of 9–71 mg/dL and an average of 32.6 mg/dL. Other complicating factors were noted in nine of our 10 hypothermia deaths including six with acute or chronic alcoholism (blood alcohol ranging from 0 to 584 mg/dL), five with heart disease, one drug toxicity, and one with diabetes.

Kanetake et al. (13) conducted a study in Japan analyzing BHB in 100 autopsies. They divided their results into five groups with ascending BHB levels using case selection and grouping criteria different from those in our study. They looked at the number of cases with conditions that were expected to generate ketones in each of the groups. The conditions included diabetes mellitus, fatty liver in alcoholics, malnutrition, and infections. In their group with the highest BHB level, 100% had ketogenic conditions, followed by 73%, 43%, and 33% in the next descending levels of BHB. In the group with the lowest BHB, only 5% had ketogenic conditions (13).

When the BHB levels of our study of 1795 cases were resorted into groups as defined by Kanetake et al. (13), our frequency distribution of ketogenic conditions resembled theirs in only one of their five groups. In the Kanetake group with the highest BHB (>10 mM), we had 36 cases and all of them had one of the ketogenic conditions. (This first Kanetake group corresponded to the highest 2/3 of our Group E). However, our remaining 1760 cases, when sorted into the other four Kanetake groups, had 38%, 35.3%, 34.2%, and 33.2% of cases in descending levels of BHB with one or more ketogenic conditions.

We had 710 cases in the second highest Kanetake group (13) where the BHB level was 1–10 mM and where the difference between the studies was the greatest, 73% versus 38% associated with ketogenic conditions. The lowest Kanetake group (<0.2 mM) included 64 cases, and only three (5%) had ketogenic conditions. Our study included 383 cases with BHB in the same range, and at least, 127 (33.2%) had a ketogenic condition.

Kanetake et al.'s (13) data suggested a linear relationship between BHB levels and the frequency of ketogenic conditions reaching 100% by 10 mM. Our data suggested a level plateau of 33–38% ketogenic conditions in BHB levels lower than 2 or 3 mM followed by an increase in ketogenic conditions to 100% at BHB levels above 6 mM.

Explanations for the discrepancies in these studies included markedly different populations and undoubtedly different dietary and drinking habits. The proportion and type of natural deaths that came under forensic investigation were likely not consistent. Diabetes and alcoholism were often not recorded in the medical history or on the death certificate. In spite of these differences, it appeared that at high levels, the BHB had a similar significance in our study as this one in Japan and the others. At the not-as-high levels of BHB, the specific characteristics of the divergent populations influenced the frequency and conditions that caused elevation of the BHB.

TABLE 4—*Recommendations for interpreting beta-hydroxybutyrate.*

Level of Beta-Hydroxybutyrate (mM)	Interpretation
<0.4	Normal
0.41–1.2	Slightly elevated and rarely (<1%) of concern
1.21–2.0	Moderately elevated and less rarely (2.5%) of concern
2.01–6.0	Significantly elevated and frequently (12–48%) of concern
>6.0	Almost always (100% in this study) indicated a life-threatening condition

Conclusion

Postmortem vitreous BHB levels correlated with vitreous glucose levels ($r = 0.731$), but not with blood alcohol levels. A postmortem vitreous BHB of greater than 6.0 mM ($n = 52$) was a marker of DKA when glucose was over 200 mg/dL, and it was a marker of alcoholic ketoacidosis when glucose was below 200 mg/dL.

A postmortem vitreous BHB of 2–6 mM ($n = 105$) was associated with elevated glucose levels from 12% to nearly 50% of the time. A history of diabetes was present in over 40% of these cases, and a history of alcoholism was in 20% of them. All of the cases of alcoholism at this level of BHB had glucose levels below 60 mg/dL. Severe infections were also noted in 18% of these cases. BHB was extremely helpful in sorting out these more difficult cases.

A postmortem vitreous BHB of 1.2–2.0 ($n = 439$) although elevated was only rarely significant; 2.5% of the cases had glucose elevated above 200 mg/dL consistent with DKA. Postmortem vitreous BHB below 1.2 mM ($n = 1199$) was even less frequently (<1%) associated with an elevated glucose level and DKA. A medical history of diabetes was noted in 14–16% of all of the cases with a BHB level <2 mM, and a history of alcoholism was noted in 8–12% of them. The usefulness of a BHB level in this lower range was usually to not ascribe these deaths to ketoacidosis without other compelling findings.

This study was generally consistent when compared with other studies. The variability both within this study and between this study and others renders conclusions tentative. Based on the results of the analysis of the 1795 cases in this study with consideration of the recommendations made in other studies (10–13), the recommendations for interpreting postmortem vitreous BHB levels are offered in Table 4.

BHB in blood was the clinical laboratory test of choice to evaluate ketoacidosis at our large county hospital that serves our frequently obese and racially diverse population with an enormous burden of diabetes. Vitreous fluid was easier to collect than blood in the postmortem setting, and hemolysis was less of a problem. Vitreous BHB was easy and inexpensive for the laboratory to run. In our setting, the cost was nominal in comparison with any other test or laboratory we might chose. Vitreous BHB has been extremely helpful when evaluating cases with suspected or known ketogenic conditions, especially diabetes and alcoholism.

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