CASE REPORT

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Postmortem Diagnosis of Unsuspected Diabetes Mellitus Established by Determination of Decedent's Hemoglobin A1c Level

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ABSTRACT: Although approximately 15.7 million Americans have diabetes mellitus, with the vast majority having type 2 diabetes, it is estimated that as many as 5.4 million are undiagnosed. The present case illustrates that undiagnosed diabetes can be a factor in otherwise unexplained deaths. A 39-year-old white male with no significant past medical history other than alcohol abuse was found deceased at his residence. The manner of death appeared to be natural, but no anatomic cause was found. Toxicological analysis revealed a blood ethanol level of 0.02 g/dL and was negative for drugs of abuse. Analysis of the vitreous fluid revealed a glucose level of 502 mg/dL. The blood glucose level was 499 mg/dL, and the hemoglobin A1c (HbA1c) level was 10.6%. Only trace urine ketones were detected, suggesting that the death was the result of hyperglycemic hyperosmolar non-ketosis (HHNK) from unsuspected diabetes. The postmortem HbA1c value serves as a definitive indicator of prolonged hyperglycemia. In order to aid the interpretation of the clinical data, this case is discussed in conjunction with a similar case of a known diabetic patient.

KEYWORDS: forensic science, hemoglobin A1c, diabetes

Of approximately 15.7 million Americans with diabetes, it is estimated that 5.4 million are undiagnosed. Diabetes is also under-reported on death certificates, both as an illness and as a cause of death (1). One of the reasons for this is that postmortem blood glucose levels are difficult to interpret. As a result, vitreous humor has become a popular specimen for analysis of glucose. For the postmortem diagnosis of diabetic ketoacidosis (DKA), elevated vitreous humor glucose and the presence of ketones may be sufficient (2). Glucose in the vitreous humor, however, is also subject to glycolysis and in the case of hyperglycemic hyperosmolar non-ketosis (HHNK) found in type 2 diabetes, increased ketones may not be present.

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Criteria for the diagnosis of diabetes mellitus in living patients have been debated. Currently, the most commonly used screening test is a fasting serum glucose level. It has been argued, however, that long-term markers of blood glucose levels might be more suitable as screening tests. Since hemoglobin A1c (HbA1c) is a marker of blood glucose control over a two-three month period and is routinely used as a guide for therapeutic management, it has been proposed as a diagnostic tool as well as a management tool (3–9). HbA1c is formed as a stable ketoamine from the non-enzymatic condensation of glucose with the N-terminal valine of the Beta-chain of hemoglobin. Once formed, HbA1c is fairly stable and thus has also been proposed as an aid in the postmortem diagnosis of diabetes (10–14).

We present the case of a 39-year-old white male with no significant past medical history other than alcohol abuse who had loss of appetite and polydipsia for approximately one to two weeks prior to his death. Postmortem evaluation, including a HbA1c level of 10.6%, suggested that the patient suffered from hyperglycemic hyperosmolar non-ketosis due to unsuspected diabetes. This case is also compared to a similar one in which the decedent was known to have had diabetes.

Case Histories

Case 1

Case 1 was a 39-year-old white male with no significant past medical history who was found deceased in his home. According to the history obtained from the relative who found him, the decedent had loss of appetite for approximately one and a half weeks and was consuming large quantities of liquids. He had considered seeking medical attention but had not done so. On the date of his death, he reportedly complained that he could not move his arms or legs. He continued to drink large amounts of liquid and was found dead approximately 12 h later. The decedent had no known medical illnesses and was not taking any medications. The relative did report that the decedent had a history of abusing alcohol until approximately four months prior to his death (although average daily consumption was not specified). The decedent also smoked one pack of cigarettes per day. There was no history of illicit drug use or recent trauma.

Case 2

Case 2 was a 45-year-old white female with a 10-year history of "brittle" diabetes (gestational-onset) who was found deceased in her home approximately 8 h after she was last seen alive. The decedent was reportedly in her usual state of health without complaints of illness or pain. The decedent was found on the floor of her bedroom without signs of trauma. She reportedly did not smoke or consume alcoholic beverages.

Postmortem Gross Findings

Postmortem examination in each case consisted of external and internal examination with collection of biological fluids, including blood, urine, bile, and vitreous humor for toxicological analyses. Findings in both cases were similar, with no clear anatomic cause of death. Severe visceral congestion, including pulmonary congestion and edema, with a mildly fatty liver and myocardial dilatation was described in Case 1. Similarly, in Case 2, moderate visceral congestion with pulmonary congestion and edema was described although no fatty liver was observed.

Routine Laboratory Analyses

Toxicological analyses were performed on the postmortem blood and urine from both cases. For drugs of abuse, a combination of immunoassays (Emit II, Syva/Behring Diagnostics, Inc., Cupertino, CA) and thin layer chromatography (Toxilab™, Toxi-Lab, Inc., Irvine, CA) were used to screen, followed by confirmatory gas chromatography with mass spectrometry (GC/MS) (15). For the analysis of volatile compounds, including ethanol and acetone, headspace analyses on GC was performed (16).

Vitreous humor and blood electrolytes were measured using ion specific electrodes on automated analyzers. Vitreous humor and blood from Case 1 were analyzed on a Beckman Synchron LX-20 auto-analyzer (Beckman Instruments, Inc., Fullerton, CA). Vitreous humor from Case 2 was analyzed on a Hitachi 747 auto-analyzer (Boehringer Mannheim Corporation, Indianapolis, IN). Blood from Case 2 was analyzed on a Dimension XL (Dupont, Wilmington, DE). Urinary ketones were measured using Multistix® 9 (Bayer Corporation Diagnostics Division, Elkhart, IN).

Hemoglobin A1c (HbA1c) Analyses

Blood was collected from the aortas of the two decedents and placed in containers along with appropriate quantities of sodium fluoride. Aliquots from samples in each case were obtained for HbA1c analyses. Hemoglobin A1c analyses were performed by high performance liquid chromatography on the Tosoh A1c 2.2 Plus Glycohemoglobin Analyzer (Tosoh Medics, Foster City, CA) according to the manufacturer's instructions. The Tosoh analyzer uses a non-porous HPLC ion exchange column in order to separate various hemoglobin species, which are quantitated spectrophotometrically by an optical detector as they elute from the column. Samples run on the Tosoh HPLC require no pre-treatment step in contrast to immunoassay methodology. The instrument withdraws a sample directly from the tube and all processing of the sample is performed internally. Prior to determination of any patient HbA1c values by HPLC, linearity of the Tosoh analyzer was determined over a range of 5.28% to 16.97%, using five standards, each run in quadruplicate. Blood samples from twelve other forensic cases were used to establish baseline postmortem HbA1c levels. All postmortem samples were from decedents were less than 55 years of age with known causes of death (gun shot wounds, motor vehicle accidents, etc). None of these control subjects had any history suggestive of diabetes mellitus.

Results

Results from the laboratory analyses of the two study cases are shown in Table 1. Toxicological analysis of the postmortem blood sample from Case 1 revealed an ethanol level of 0.02 g/dL (analysis of the urine and vitreous humor was negative for ethanol). The urine in Case 1 was negative for drugs of abuse. The amount of ethanol detected within the blood in this case may possibly have been the result of postmortem fermentation. Since the ocular fluid glucose and potassium levels were 502 mg/dL and 8.0 mmol/L, respectively, the blood was retrospectively analyzed to reveal a blood glucose level of 499 mg/dL. Only trace levels of ketones were observed in the urine. HbA1c analysis subsequently yielded a value of 10.6%. In comparison, the baseline HbA1c levels obtained from the non-diabetic decedents ranged from 4.6-6.8% (Table 2).

Toxicological analysis of the postmortem blood and urine from Case 2 did not reveal the presence of ethanol or drugs of abuse. The vitreous humor potassium level was elevated in this case, most likely the result of autolysis. The blood and ocular fluid glucose levels, however, were not apparently significantly elevated. The HbA1c level was 9.0%.

Figure 1 shows the Tosoh HPLC chromatograms from a non-diabetic control patient and the two decedents. The Y-axis shows the percentage of hemoglobin with a scale of up to 15%. The x-axis shows the time elapsed in minutes for hemoglobin elution from the column. The shaded peak is the stable HbA1c fraction. The smaller peaks correspond to hemoglobin F and labile hemoglobin fractions that elute earlier than the stable hemoglobin A1c fraction, as shown. The largest peak is hemoglobin A, which elutes at 1.92 min. Total analysis time is 3 min per sample. The instrument calculates

TABLE 1—*Laboratory analyses of decedents' biological fluids. Case 1—The decedent had a markedly elevated postmortem glucose level both in his vitreous humor and blood. His HbA1c level was also markedly elevated. Since only trace ketones were present in the urine, a diagnosis of hyperglycemic hyperosmolar non-ketosis is more likely than diabetic ketoacidosis. A known diabetic, Case 2, had similar postmortem findings. (NA—not applicable.)*

	Case 1			Case 2		
	Vitreous	Blood	Urine	Vitreous	Blood	Urine
K^+ (mmol/L)	8	NA		8.6	NA	
Glucose (mg/dL)	502	499		20	170	
HbA1c (%)		10.6			9.0	
Ethanol (g/dL)	Negative	0.02	Negative		Negative	
Drug screen			Negative			Negative
ketones			Trace			

TABLE 2—*Control cases where the decedents had no known history of diabetes were used to establish baseline postmortem HbA1c levels. The decedents ranged from 16-54 years of age. The HbA1c levels ranged from 4.6%–6.8%. None had hemoglobin variants. (M-male; F-female; GSW-gun shot wound; MVA-motor vehicle accident.)*

Case No.	HbA1c (%)	Age, Sex, and Cause of Death
3	6.1	49 M Tornado Victim
4	5.7	27 F Tornado Victim
5	6.2	37 M Tornado Victim
6	4.6	21 M GSW
7	6.8	33 M GSW
8	6.8	54 M GSW
9	6.8	18 M MVA
10	6.3	16 M GSW
11	6.1	25 M Industrial Accident
12	5.4	46 M GSW
13	6.4	25 M GSW
14	5.1	23 M Natural

the percentage of HbA1c by integrating the area under the shaded peak, dividing that area by the total integrated areas under all the peaks that elute prior to 2 min, and multiplying the result by 100%.

Discussion

The two cases discussed above illustrate how HbA1c levels can be used to confirm a diagnosis of diabetes mellitus in the postmortem setting. Especially in Case 1, a markedly elevated HbA1c level of 10.6% was instrumental in determining the cause of death.

Hemoglobin A1c is routinely used for monitoring diabetic patients, as it is a marker of blood glucose control over the preceding 2–3 months. However, the use of HbA1c in the diagnosis of diabetes (3–9) and in the postmortem diagnosis of diabetes has been controversial (10–14). The controversy surrounds the cutoff level to be used to make the diagnosis. In clinical studies, Peters et al. suggested a HbA1c level of 7% or higher (3) while Davidson et al. suggested a glycated hemoglobin level greater than or equal to 1% above the upper limit of normal (4). For the postmortem diagnosis of diabetes, few studies have been done. In a small study, Chen et al. showed that postmortem HbA1c of five decedents with a history of diabetes were elevated, with values that may be seen in patients with poorly controlled diabetes. The mean HbA1c value of decedents without the history of diabetes was higher than that of "normal volunteers" (10). Hindle et al. showed that HbA1c levels were elevated in eight diabetic decedents with HbA1c ranging from 8.7–15.4%. In some instances, normal HbA1c levels were seen in the presence of grossly elevated postmortem glucose levels. Hindle et al. concluded that HbA1c levels are more reliable than postmortem glucose levels in cases where diabetes as a contributing factor in death is suspected (11). For this reason, the aforementioned result should be used in conjunction with blood and ocular fluid glucose and electrolytes in the final determination of the cause of death, specifically in cases where diabetes is suspected.

The aforementioned studies were performed when methodologies for HbA1c analysis were technically difficult and cumbersome. Also, since HbA1c reflects blood glucose levels over a 2–3 month period, it is not necessarily indicative of the blood glucose level during the period of time surrounding death. Some investigators have attempted to use markers of short-term blood glucose control, such as glycated serum proteins (12–14,17–18) to make

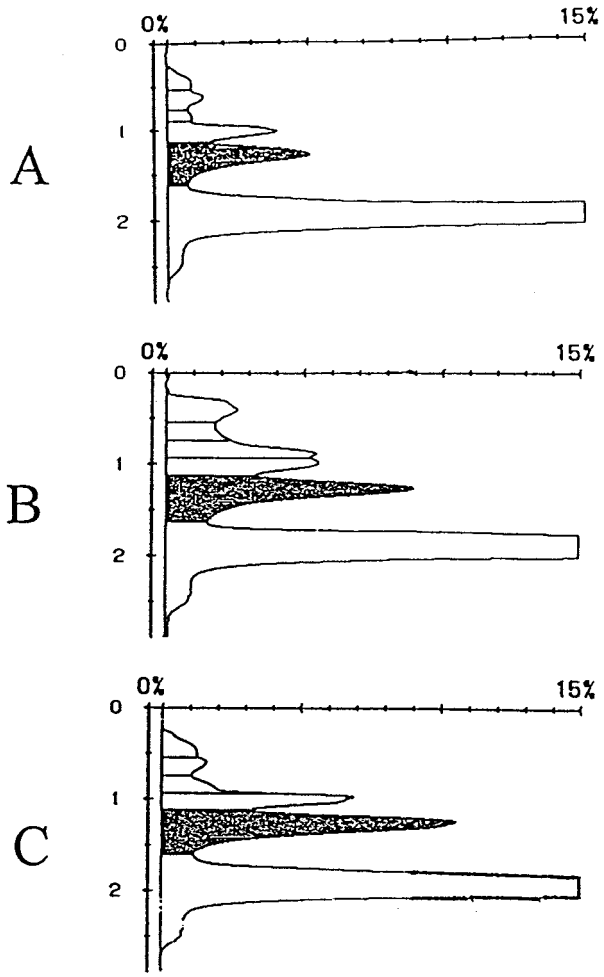

FIG. 1—*HPLC chromatograms of postmortem HbA1c levels of a non-diabetic patient, a known diabetic patient (Case 2), and an unsuspected diabetic patient (Case 1). A—a representative chromatogram from a non-diabetic control patient with a HbA1c level of 5.1%. The y-axis shows the percentage of hemoglobin. The x-axis shows the time of elution from the column. Hemoglobin F and labile hemoglobin A1c fractions appear elute earlier than the stable hemoglobin A1c fraction. The shaded peak is the stable HbA1c fraction. The last and largest peak is hemoglobin A, which elutes around 1.92 min. The instrument calculates the percentage of HbA1c by integrating the area under the shaded peak, dividing that area by the total integrated areas under all the peaks that elute prior to 2 min, and multiplying the result by 100%. The smaller peaks correspond to hemoglobin F and labile hemoglobin fractions. B—Chromatogram from a known diabetic patient (Case 2) with a postmortem HbA1c level of 9.0%. C—Chromatogram from an unsuspected diabetic patient (Case 1) with a postmortem HbA1c level of 10.6%.*

that determination. Serum fructosamine has been proposed as a potential marker since it has a significantly shorter half-life than HbA1c. Unfortunately, in the postmortem setting, fructosamine can be technically difficult to measure since many postmortem samples are hemolyzed. In contrast, Chen et al. showed that HbA1c is stable for at least 36 h after death (10). With the availability of automated HPLC methodologies, measurement of HbA1c can be performed with ease and will probably gain popularity in the forensic arena, just as it has in the clinical setting. The advantage of the HPLC method for measuring HbA1c is that it also serves to screen for hemoglobin variants. Where immunoassays will report "HbA1c" results in cases where the patient may not have hemoglobin A (SS, SC, etc.), the HPLC method will not register a

result when the appropriate amount of hemoglobin A is not detected.

The decedent in Case 1 was hyperglycemic at the time of death as reflected by the elevated vitreous glucose level. The decedent's HbA1c level demonstrated that he had been hyperglycemic for some time prior to his death. In the absence of increased ketones, his death appears to have resulted from hyperglycemic hyperosmolar non-ketosis due to undiagnosed diabetes. Analysis of the HbA1c level was clearly useful in establishing the cause of death in this case. In our opinion, HbA1c testing is an important adjunct for the postmortem diagnosis of diabetes mellitus and the determination of the cause of death in certain selected cases.

References

1. Report of the Expert Committee on the diagnosis and classification of diabetes mellitus. Diabetes Care 1997 July;20(7):1183–97.
2. Coe JI. Postmortem chemistry update: Emphasis on forensic application. Am J Forensic Med Pathol 1993;14(2):91–117.
3. Peters AL, Davidson MB, Schriger DL, Hasselblad V. A Clinical approach for the diagnosis of diabetes mellitus: An analysis using glycosylated hemoglobin levels [see comments] [published erratum appears in JAMA 1997 Apr;277(14):1125]. JAMA 1996 Oct;276(15):1246–52. Comment in: JAMA 1997 Jan;277(3):211.
4. McCance DR, Hanson RL, Charles M, Jacobsson LT, Pettitt DJ, Bennett PH, et al. Comparison of tests for glycated hemoglobin and fasting and two hour plasma glucose concentrations as diagnostic methods for diabetes [see comments]. Br Med J 1994 May;308(6940):1323–28. Comment in: Br Med J 1994 August;309:537–8.
5. Davidson MB, Peters AL, Schriger DL. An alternative approach to the diagnosis of diabetes with a review of the literature. Diabetes Care 1995 July;18(7):1065–71.
6. Singer DE, Coley CM, Samet JH, Nathan DM. Tests of glycemia in diabetes mellitus. Their use in establishing a diagnosis and in treatment. Ann Intern Med 1989 Jan;110(2):125–37.
7. Lester E. The clinical value of glycated haemoglobin and glycated plasma proteins. Ann Clin Biochem 1989;26:213–9.
8. Saleman TH, van Dieijen-Visser MP, Brombacher PJ. The value of HbA1 and fructosamine in predicting impaired glucose tolerance-an alternative to OGTT to detect diabetes mellitus or gestational diabetes. Ann Clin Biochem 1987;24:447–52.
9. Gerken KL, van Lente F. Effectiveness of screening for diabetes. Arch Pathol Lab Med 1990;114:201–3.
10. Chen C, Glagov S, Mako M, Rochman H, Rubenstein AH. Postmortem glycosylated hemoglobin (HbA1c): Evidence for a history of diabetes mellitus. Ann Clin Lab Sci 1983;13(5):407–10.
11. Hindle EJ, Rostron GM, Gatt JA. The diagnostic value of glycated hemoglobin levels in postmortem blood. Ann Clin Biochem 1985; 22:144–7.
12. Akane A, Shiono H, Matsubara K, Tanabe K, Nakamura H, Hasegawa M, et al. Analysis of glycated albumin in postmortem blood samples as the diagnostic parameters of diabetes mellitus. Jpn J Legal Med 1992; 46(4):237–43.
13. Valenzuela A. Postmortem diagnosis of diabetes mellitus. Quantitation of fructosamine and glycated hemoglobin. Forensic Sci Int 1988; 38:203–8.
14. John WG, Scott KWM, Hawcroft DM. Glycated haemoglobin and glycated protein and glucose concentrations in necropsy blood samples. J Clin Pathol 1988;41:415–8.
15. Foerster EH, Hatchett D, Garriott JC. A rapid, comprehensive screening procedure for basic drugs in blood or tissues by gas chromatography. J Anal Toxicol 1978;2:50–5.
16. Karnitis L, Porter LJ. A gas chromatographic method for ethanol in vapors of biological fluids. J Forensic Sci 1971;16:318–22.
17. Maruna P, Skrha J, Strejc P. Serum fructosamine after death. Diabetic Med 1989;6:460.
18. Ritz S, Mehlan G, Martz W. Postmortem diagnosis of diabetic metabolic derangement: Elevated alpha-1 antitrypsin and haptoglobin glycosylation levels as an index of antemortem hyperglycemia. J Forensic Sci 1996 Jan;41(1):94–100.

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