J Forensic Sci, Nov. 2002, Vol. 47, No. 6
Paper ID JFS2001395_476
Available online at: www.astm.org

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HbA1c as a Postmortem Tool to Identify Glycemic Control*

ABSTRACT: Estimates suggest that more than 5.4 million U.S. citizens unknowingly have diabetes and are at increased risk of morbidity and mortality. We evaluated an immunoturbidimetric measurement of glycated hemoglobin (%HbA1c) as a postmortem tool to identify such individuals. Although postmortem samples undergo some degradation, the effects are not sufficient to invalidate the use of the test or method. Using two study populations whose medical history of diabetes was known, we found the mean %HbA1c of the non-diabetics (5.8 ± 0.3) to be statistically different from that of the diabetics (12.4 ± 2.8). For the population whose disease status was unknown, the %HbA1c ranged from 4.7 to 16.8. For six unknowns whose values exceeded 7.0%, the mean was 11.7%, which did not differ statistically from the diabetic mean ($p = 0.6615$). These studies suggest that postmortem blood samples can be used to characterize HbA1c values.

KEYWORDS: forensic science, laboratory medicine, postmortem blood, glycated hemoglobin, diabetes mellitus

Diabetes mellitus is a serious medical condition in which the metabolic use of glucose is impaired, resulting in chronic hyperglycemia. Despite increasing public awareness of and available treatments for the disease, over 190 000 deaths are attributed annually to diabetes (1). Unfortunately, when investigating these situations, medical examiners find the measurement of glucose in postmortem blood an unreliable indicator of antemortem glycemic control (2). This is because several normal and pathological events may occur, causing a transient hyperglycemic state. These include the recent ingestion of food, infusion of intravenous glucose or parenteral nutrition supplements, administration of some medications, or the loss of islet cell control as occurs in some cases of acute pancreatitis or head trauma (3,4). It has also been demonstrated that an increase in glucose may occur as an "agonal alarm reaction" in association with an increased release of catecholamines (5). Lund determined that the amount of glucose in the periphery from this response depends upon the agonal situation and its duration (5). Additionally, the administration of epinephrine during cardiopulmonary resuscitative attempts to revive the victim may further enhance glycogen mobilization and the manual chest compression may artificially pump the mobilized glycogen and glucose through the circulation (6). On the opposing side, cellular and bacterial utilization of existing glucose continues several hours after death. As a result, the remaining glucose may be much less than at the time of death.

Because of these complicating factors, alternatives to the measurement of glucose in blood have been sought when investigating deaths in which diabetes may have played a role. These have included the measurement of other analytes and the use of other body fluids such as vitreous humor and cerebrospinal fluid (CSF). Acetone, measured in blood, vitreous, or CSF, is also increased in diabetic ketoacidosis. But as with glucose, increased concentrations of this analyte may also result from other situations, including isopropyl alcohol ingestion, hypothermia, severe liver disease, starvation, and some diets. While increased glucose concentration in vitreous humor reflects hyperglycemia at the time of death, this measurement is not without controversy and unless the medical condition is considered at the time of autopsy, vitreous samples may not be collected. Glucose metabolism may occur in both vitreous and CSF, so several investigators have suggested the use of additional analytes such as lactate, fructosamine, and microalbumin (2, 5–11). Schemes to correct for metabolic loss include the summing of glucose and lactate concentrations, or the use of ratios such as glucose/lactate or glucose/fructosamine, etc.

The measurement of glycated hemoglobin is an effective tool in monitoring long-term glucose control in people with diabetes because it represents an "average" blood glucose level for the preceding 6–8 weeks. We evaluated the utility of measuring the percentage of glycated hemoglobin $A1_c$ (HbA1c) in blood samples collected postmortem as a means of assessing glycemic control during the 6–8 weeks preceding death. If elevated, these determinations could implicate diabetes as a contributing factor in a natural death. This is not the first evaluation of glycated hemoglobin for this purpose, but it encompasses a much larger number of samples than previous studies and case reports (12–15). Additionally, it is the first to use an immunological-based method and to address issues of specimen stability.

Experimental

Methods of Analysis

The %HbA1c of 76 postmortem whole blood specimens was measured in singlecate using a turbidimetric immunoinhibition-

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* Presented at the Academy of Forensic Sciences, Seattle, Washington, February 2001 and at the American Association for Clinical Chemistry, Chicago, Illinois, July 2001.
Received 29 Nov. 2001; and in revised form 2 May 2002; accepted 28 May 2002; published 18 Sept. 2002.

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based method on a Hitachi 911 analyzer (both from Roche Diagnostics Corporation, Indianapolis, IN). The analysts were blind to the status of the specimens. Prior to analysis, samples were mixed using a rocking-device for approximately 15 min, and 10 μL of specimen was pipetted into a labeled sample cup and 1.0 mL of hemolyzing reagent (0.9% tetradecyltrimethylammonium bromide, Sigma-Aldrich, St. Louis, MO) was added to the cup and mixed. After a two-minute incubation the specimens were transferred to the autoanalyzer. The method is based on the formation of soluble antigen-antibody complexes between an antibody to HbA1c in the reagent and glycated hemoglobin in hemolyzed whole blood specimens. Excess antibody reacts with polyhaptens contained in a second reagent, resulting in the formation of agglutinated complexes. The complexes are measured turbidimetrically and the amount of turbidity is inversely proportional to the amount of HbA1c in the specimen. The amount of hemoglobin in the specimen is measured photometrically and the %HbA1c is calculated automatically. For this method, the observed imprecision (%CV) using control material (Roche Diagnostics, Indianapolis, IN) is 2.5% at 6.7% HbA1c and 2.0% at 10.9% HbA1c. The reference range for normoglycemic adults is 4.8–6.0% HbA1c for this assay.

Acetone was quantified in postmortem specimens using headspace gas chromatography as part of the volatile assay performed routinely at the Office of the Chief Medical Examiner (Chapel Hill, NC). A single point with acetone as the calibrator (100 mg/dL) and n-propanol (100 mg/dL) as the internal standard was used for calibration. Analytical grade acetone and *n*-propanol were purchased from Fisher Scientific (Pittsburg, PA). The instrumentation included an HS-40 autosampler (Perkin-Elmer Corporation, Shelton, CT) and Varian 3800 gas chromatograph equipped with flame ionization detectors (Varian Corporation, Walnut Creek, CA). The specimen (100 μL) was introduced into a 20 mL headspace vial with internal standard (100 μL) and sealed. After equilibration on the autosampler (60°C for 10 min), the headspace vapor was sampled and split between two analytical columns, a 0.53 mm × 3.0 μm × 30m Rtx-BAC1 and a 0.53 mm × 3.0 μm × 30 m Rtx-BAC2 (Restek Corporation, Bellefonte, PA) for an isothermal analysis at 35°C and a run time of 4 min.

Postmortem Samples

Seventy-six postmortem whole blood specimens were selected for HbA1c analysis. Samples were selected using the following criteria: (1) the absence of a history of diabetes preceding death due to accident or suicide, (2) the history of diabetes and a postmortem request for acetone analysis, (3) the presence of acetone following the routine volatile analysis. For each of the selected samples, case files were reviewed for medical history, particularly for evidence of diabetes type 1 or type 2; and, for some cases, additional medical history was sought.

These samples were collected at the time of autopsy or medical examiner investigation in standard autopsy collection vials (20 mL polypropylene vials with 2% NaF). The postmortem interval (days from death to collection) ranged from less than 1 day to 16 days (77% of samples were collected within 2 days of death). The samples were stored at 4°C for periods from 1 to 125 days. Of the samples selected, 61 were suitable for analysis, while 15 were unsuitable.

Postmortem Population

The sample population included 11 negative controls, 21 positive controls, and 29 unknowns. A negative control was defined as a decedent without a medical history for either type of diabetes and whose manner of death was accident or suicide. For inclusion into this category, there could be no acetone detected in the postmortem specimens. A positive control was defined as a decedent whose medical history included type 1 or 2 diabetes. An unknown was defined as a decedent not known to have diabetes, and whose postmortem acetone level was > 2 mg/dL.

Stability Studies

Four studies of sample stability were conducted. Specimens are transported to the toxicology laboratory by a variety of methods including regular parcel post and during transit the specimens may be exposed to changing temperatures. In addition, decomposing bodies may experience changes in temperature. Therefore, the first stability study assessed the affect of temperature on the stability of the sample. Blood was collected from four healthy volunteers into vacuum tubes containing 5.4 mg K_2EDTA. These volunteers included two nondiabetics, one type 1 diabetic, and one type 2 diabetic. The samples were placed into the standard autopsy collection vials as described above and after initial testing, incubated at the same, but variable, temperatures (24 to 35°C) for 7 days. The time spent at each temperature was as follows: 35°C for 48 h, 30°C for 24 h and 24°C for 96 h. The samples were reanalyzed and the values compared.

Immediately preceding or following death, the blood glucose concentration is reported to reach levels up to 800 mg/dL. To simulate this situation, a concentrated glucose solution was added to five samples immediately after initial testing so that the final concentration exceeded 500 mg/dL for a second stability study. The samples were then incubated at room temperature for 7 days and reanalyzed.

A third study comparing samples collected in EDTA and NaF was also undertaken to determine if sample preservation with NaF resulted in interference with the measurement of %HbA1c. Two samples, one EDTA and one without anticoagulant or preservative, were collected from two healthy volunteers (one nondiabetic and one type 1 diabetic). The nonpreserved specimens were immediately placed into standard autopsy collection vials containing 2% NaF and the EDTA samples were placed in autopsy collection vials without NaF. The samples were tested within 2 h, then incubated at room temperature for 7 days, and reanalyzed.

The fourth study compared the reanalysis of %HbA1c of two specimens from each postmortem study population after a storage period of two years. All specimens were originally stored at 4°C for 90–120 days and then transferred to a freezer for storage (−22°C). Prior to freezing, %HbA1c testing was conducted. At the conclusion of the project, the selected specimens were thawed, reanalyzed, and the results compared.

Statistical Analysis

Statistical analyses and graphs were performed using GraphPad Prism version 3.0 Software (San Diego, CA) and MedCalc Software (Broekstraat, Belgium).

Results

Initial, post-incubation and percent changes in %HbA1c for the stability studies are presented in Tables 1–4. The percent change averaged 5% in the temperature variation study (Table 1) and 5.8% in the excess glucose study (Table 2). Using the paired t test, statistical analysis showed the initial and post-incubation HbA1c de-

TABLE 1—*Stability of %HbA1c in specimens exposed to a variable-temperature environment.*

Stability Study 1			
	HbA1c (%)		
Sample	Initial	Post-Incubation	% Change
1	5.3	5.5	3.7
2	5.8	6.2	6.8
3	6.7	7.0	4.5
4	9.7	10.2	5.2

TABLE 2—*Stability of %HbA1c in specimens augmented with excess glucose.*

Stability Study 2			
	HbA1c (%)		
Sample	Initial	Post-Incubation	% Change
1	10.8	11.0	1.9
2	13.2	13.6	3.0
3	7.3	8.0	9.5
4	5.3	5.9	11.0
5	8.1	8.4	3.7

TABLE 3—*Stability of %HbA1c in NaF vs. EDTA.*

Stability Study 3			
	HbA1c (%)		
Sample	Initial	Post-Incubation	% Change
EDTA 1	5.5	5.5	0
2	7.6	7.7	1.3
NaF 1	5.6	5.6	0
2	7.7	7.7	0

TABLE 4—*Stability of %HbA1c in postmortem specimens.*

Stability Study 4			
	HbA1c (%)		
Group	Initial	Post-Storage	% Change
Negative controls	5.4	5.4	0
	6.1	6.6	7.9
Positive controls	11.4	11.5	0.87
	11	11.5	4.4
Unknowns	10.9	10.5	−3.7
	7.5	7.6	1.3

TABLE 5—*Demographics and %HbA1c results for negative controls.*

Negative Controls (Nondiabetic)						
			Demographics			
HbA1c (%)	Acetone (mg/dL)	PI* (days)	Gender	Age (y)	History; Cause of Death	Collection Site
5.4	nd†	<1	M	34	Possible overdose	Aorta
5.4	nd	<1	M	18	Cardiac hypertrophy	Aorta
5.4	nd	<1	M	40	GSW‡; suicide	Subclavian vein
5.6	nd	2	M	80	GSW; suicide	Thoracic puncture
5.6	nd	1	M	22	MVA§	Femoral vein
5.7	nd	2	M	22	GSW; suicide	Subclavian vein
5.8	nd	<1	F	30	Chronic ETOH((abuse; MVA	Vena cava
5.9	nd	1	F	36	GSW; suicide	Subclavian vein
6.1	nd	1	M	44	BFHT¶; MVA	Subclavian vein
6.3	nd	<1	M	21	Drowning	Not identified
6.3	nd	<1	M	38	MVA	Carotid artery

* PI—postmortem interval; †nd—none detected; ‡GSW—gun shot wound; §MVA—motor vehicle accident; ((ETOH—ethanol; ¶BFHT—blunt force head trauma.

terminations for both studies to differ significantly ($p < 0.05$). Only one data pair exhibited any change in the third study (Table 3) comparing NaF and EDTA. The change was 1.3% and using the paired t test, the results did not differ significantly ($p = 0.196$). In the final study evaluating the stability of HbA1c in postmortem specimens after storage (Table 4), the percent change averaged 3%. The paired t test indicates that the initial and post storage results did not differ significantly ($p = 0.190$).

The demographics and %HbA1c results for the negative controls, positive controls and unknowns are presented in Tables 5, 6 and 7, respectively. The average age for the negative controls was 35 years (range: 18–80 years) and the average %HbA1c was 5.8 (range: 5.4–6.3%). For eight of the eleven (73%), the %HbA1c was within the normoglycemic reference range of 4.8–6.0%. Of the three cases with HbA1c's above the reference range, one was 6.1% and two were 6.3%. The two most common causes of death in this group were gunshot wound (36%) and motor vehicle accident (36%). By our definition of the population, no acetone was detected in the blood of any negative control.

Among positive controls the average age was 54 years (range: 32–80 years) and the average %HbA1c was 12.4 (range: 7.5–19.6%). The %HbA1c was above the normoglycemic refer-

TABLE 6—*Demographics and %HbA1c results for positive controls.*

Positive Controls (Diabetics)						
			Demographics			
$HbA1_C$ (%)	Acetone0 (mg/dL)	PI* (days)	Gender	Age (y)	History; Cause of Death	Collection Site
7.5	7	2	M	36	Diabetes; bowel infarction	Aorta
9.3	22	<1	F	51	Diabetes (type 1); DKA† (vitreous glucose: 759 mg/dL)	Aorta
9.4	nd‡	1	M	57	Diabetes, IHD§	Aorta
9.9	54	1	M	52	Diabetes, poor control; AMI((	Thoracic puncture
10.5	<5	6	F	80	Diabetes: IHD	Thoracic puncture
11.0	4	<1	M	66	Diabetes, IHD	Heart
11.3	<5	<1	M	62	Diabetes, IHD	Unidentified
11.3	nd	1	M	41	Diabetes, obesity, HTN¶; IHD	Subclavian vein
11.4	9	3	M	32	Diabetes; DKA, septicemia	Thoracic puncture
11.7	nd	1	F	48	Diabetes	Vena cava
12.3	7	unk**	M	65	Diabetes; GI†† bleed, DKA	Unidentified
12.3	18	16	M	49	Schizophrenia; DKA	Heart
12.5	<5	<1	F	62	Diabetes (Type II), poor control; AMI	Subclavian vein
12.9	35	1	F	42	Diabetes, poor control; DKA	Subclavian vein
13.1	8	1	M	61	Diabetes; pneumonia	Subclavian vein
13.1	3	7	M	35	Symptomatic, not treated; DKA	Aorta
13.2	55	2	M	50	Diabetes; AMI	Aorta
16.2	<5	<1	M	64	Diabetes: IHD	Thoracic puncture
16.3	32	unk	M	46	Diabetes, ETOH†† abuse; DKA	Jugular vein
16.5	3	1	M	51	Diabetes; DKA (vitreous glucose: 1281 mg/dL)	Subclavian vein
19.6	5	1	F	78	Diabetes; MVA§§, DKA (vitreous glucose: 1111 mg/dL)	Aorta

* PI—postmortem interval; †DKA—diabetic ketoacidosis; ‡nd—none detected; §IHD—ischemic heart disease; ((AMI—acute myocardial infarction; ¶HTN—hypertension; **unk—unknown; ††GI—gastrointestinal; ‡‡ETOH—ethanol; §§MVA—motor vehicle accident.

TABLE 7—*Demographics and %HbA1c results for cases where diabetes status was unknown.*

Unknowns						
			Demographics			
HbA1c (%)	Acetone (mg/dL)	PI* (days)	Gender	Age (y)	History; Cause of Death	Collection Site
4.7	5	<1	F	<1	Congenital heart disease	Heart
4.7	<5	1	M	45	ETOH† abuse, liver disease	Aorta
5.0	8	1	M	57	GI‡ bleed	Aorta
5.1	5	unk§	F	100	IHD((	Unidentified
5.3	<5	<1	M	45	Malnutrition, ETOH abuse, flu-like symptoms	Vena cava
5.3	5	<1	M	48	ETOH abuse, CAD¶, liver disease	Vena cava
5.4	19	2	M	57	ETOH abuse, dehydration	Heart
5.5	5	1	M	35	ETOH, IVDA**, acute pancreatitis	Aorta
5.6	6	<1	M	86	Accident; chest compression	Thoracic puncture
5.6	11	2	M	70	ETOH abuse; IHD	Aorta
5.6	<5	1	M	59	ETOH abuse; CAD	Vena cava
5.7	5	8	F	71	IHD; CHF††	Subclavian vein
5.8	5	1	M	39	ETOH abuse; seizure	Jugular
5.8	11	<1	M	91	CAD	Aorta
5.8	<5	unk	M	67	Acute renal failure	Unidentified
5.9	3	1	M	36	Heroin overdose	Aorta
6.0	11	1	M	54	Cocaine use; cardiomyopathy	Iliac
6.2	14	unk	M	74	Hypothermia	Unidentified
6.2	12	<1	M	86	IHD	Subclavian vein
6.3	5	2	F	89	Possible malnutrition; senility	Subclavian vein
6.4	2	<1	M	70	IHD; AMI‡‡	Heart
6.5	2	<1	M	54	ETOH, IHD	Subclavian vein
6.9	14	4	F	83	CHF	Heart
7.8	26	unk	M	42	Peritonitus, pancreatitis	Unidentified
9.3	20	unk	F	82	COPD§§; possible malnutrition	Unidentified
10.6	7	<1	M	49	AMI	Jugular
10.9	39	<1	F	60	CAD; pericarditis	Heart
14.8	3	unk	F	64	AMI	Unidentified
16.8	<5	<1	M	57	No medical hx; pneumonia	Aorta

* PI—postmortem interval; †ETOH—ethanol; ‡GI—gastrointestinal; §unk—unknown; ((IHD—ischemic heart disease; ¶CAD—coronary artery disease; **IVDA—intravenous drug abuse; ††CHF—congestive heart failure; ‡‡AMI—acute myocardial infarction; §§COPD—chronic obstructive pulmonary disease.

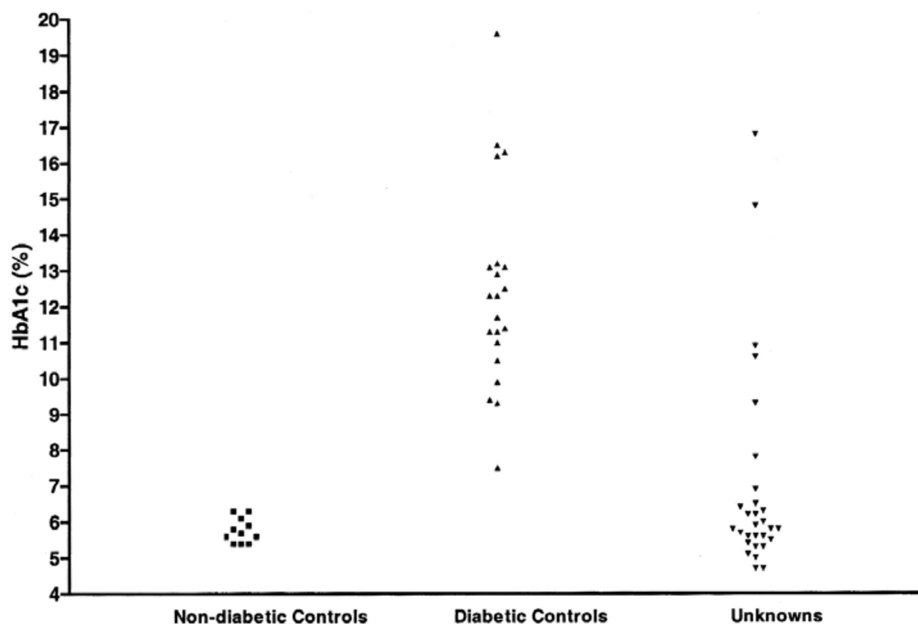

FIG. 1—*Comparison of %HbA1c between groups. Nondiabetic controls (n = 11) defined as a decedent without a medical history of diabetes. Diabetic controls (n = 21) defined as a decedent whose medical history included a diagnosis of diabetes. Unknowns (n = 29) defined as a decedent whose diabetes status was not known and whose postmortem acetone was > 2 mg/dL.*

ence range for all subjects within this population. Vitreous specimens were collected and analyzed for glucose for three of the positive controls. The results of the glucose analyses are detailed in Table 6. The two most common causes of death in this group were diabetic ketoacidosis (48%) followed by ischemic heart disease (28%). Acetone was detected in 86% of the cases and averaged 13 mg/dL (range: 0–55 mg/dL). The %HbA1c for this group differed statistically from the negative controls at $p < 0.0001$.

The average age for the unknowns group was 61 years (range: < 1–100 years) and the average %HbA1c was 6.9 (range: 4.7–16.8%). The two most common causes of death in this group were ischemic heart disease (28%) followed by coronary artery disease (21%). Seventeen of 29 (59%) had %HbA1c's within the normoglycemic reference range. For this subgroup, acetone concentrations ranged from 2 to 11 mg/dL (mean = 6.5 mg/dL).

The subgroup of unknowns with HbA1c values above the reference range (6.0%) had an average HbA1c of 9% (range: 6.2–16.8%). While the %HbA1c for this group differed statistically from the negative controls at $p < 0.0001$, this parameter for the group did not differ statistically from the diabetic controls. For this subgroup, acetone averaged 12.3 mg/dL (range: 2–39 mg/dL).

Figure 1 illustrates and contrasts the range and grouping of %HbA1c among the negative controls, positive controls and unknowns. The negative controls exhibit a tight grouping between 5.5 and 6.5% whereas the diabetic controls are spread throughout the hyperglycemic range (>7%). The unknowns exhibit a pattern that encompasses both the patterns for the negative and the positive controls.

Discussion

Previous studies investigating the utility of glycated hemoglobin as a marker of glycemic control in postmortem samples have limitations including time consuming methodologies, lack of method evaluation for differentiating between positive and negative controls, and/or small sample size (11–14). Valenzuela utilized gel electrophoresis, Tracy et al. utilized cation exchange column chromatography, and John et al. utilized both gel electrophoresis and affinity chromatography for the measurement of HbA1c. Valenzuela focused mainly on the use of fructosamine as an estimate of peri-mortem glucose levels and found it useful in diagnosis, whereas John et al. found that most of their postmortem specimens were unsuitable for fructosamine testing due to hemolysis. Tracy et al. analyzed glycated hemoglobin as part of a large study investigating the role of nephrosclerosis, glycohemoglobin, cholesterol, and smoking in coronary heart disease deaths. Due to the nature of this investigation, this study excluded known diabetics from the statistical analyses. All three of these studies utilized methods of analysis that are labor intensive and time consuming and none of these studies focused on the use of HbA1c for postmortem diagnosis of suspected diabetics. The only study that concentrated on undiagnosed diabetics was a case report by Khuu et al. This study tested postmortem blood from a small population consisting of one undiagnosed or suspected diabetic, one known diabetic and 14 negative controls.

Although all of these investigations have suggested HbA1c can be measured in postmortem samples, a review of the literature could find no evidence of confirmation that HbA1c is stable under the conditions encountered with medical examiner samples. Also, since there are situations immediately preceding or following death during which glucose is released, stability studies were included with this investigation to determine if a normoglycemic individual could be misclassified as chronically hyperglycemic and thus potentially diabetic.

These studies demonstrate that the %HbA1c can increase after collection and as with previous clinical studies this study observed that the increases seen are almost exclusively due to changes in the hemoglobin value (16,17). When the temperature was varied, the %HbA1c was found to increase from 4–7% above the initial values. Similarly, the addition of excess glucose increased the post-incubation results by 2–11%. Despite an insignificant change to the overall clinical picture, statistical analysis by the paired t test,

showed the initial and post-incubation HbA1c determinations for both studies to differ significantly ($p < 0.05$).

With respect to the varying temperature conditions, the results are similar to those encountered with EDTA anticoagulated venous and capillary samples used for patient care. Recently, many clinical facilities adopted the use of capillary specimens to facilitate testing. These samples are collected at home and shipped to the testing laboratory via surface mail. Initially there were concerns that the range of conditions (temperature, most importantly) would adversely impact sample integrity. Preliminary and subsequent studies have demonstrated that degradation can occur under extreme conditions, but most samples are testable. Several studies have shown that when these clinical samples are exposed to fluctuating temperature over periods of 7–10 days, post-exposure results increase by 8–9% compared to samples tested within 24 h of collection and stored at 4°C (16,17). In these cases, there is not the additional formation of HbA1c. Rather the apparent increase is most likely related to hemoglobin degradation. The authors concluded that while the changes in %HbA1c were statistically significant, the changes did not alter the clinical interpretation. However, it remains that these studies and the present study show that if the %HbA1c is just at the upper limit of the normoglycemic reference range, exposure to these conditions could raise the result above the range. In the clinical setting, such a finding would prompt repeat collection and testing. In contrast, the last two stability studies exhibited minimal changes in HbA1c and we were able to confirm that NaF does not interfere in HbA1c analysis and the postmortem specimens are stable under common storage conditions.

Regarding the suitability of a sample for analysis using this method, two observations should be mentioned. First, some of the unsuitable samples were noted to contain small clots, while other samples appeared "diluted" having most likely been contaminated with tissue fluids. There was no correlation between the length of the postmortem interval and sample integrity. For either type of sample an accurate measurement of hemoglobin would not be possible. This would be the case with any of the HbA1c methods currently available.

The range of %HbA1c for the negative (nondiabetic) controls was found to be 5.4 to 6.3% (mean 5.8% $\pm$ 0.34). This range is slightly higher than the reference range determined by our clinical laboratory for normoglycemic adults and may represent increases related to storage and stability as just discussed. The %HbA1c for all decedents previously identified as diabetic exceed this range: 7.5 to 19.6% (mean 12.4% $\pm$ 2.8). These results are consistent with the histories that most of these individuals were in poor glycemic control and/or that diabetes was a contributing factor in their death. With respect to %HbA1c, the negative controls were found to differ statistically from the diabetic group at $p < 0.0001$. The range of acetone concentrations found within these two groups further emphasizes the limitations of this analyte for the purpose of assessing glycemic control. By definition, there was no acetone found in the postmortem blood samples of the negative controls. For the diabetic controls, acetone ranged from none detected to 55 mg/dL. More importantly, there are several cases in which some of the lowest measurable levels (3–5 mg/dL) were observed in conjunction with the higher %HbA1c levels observed.

The results for the unknown group range from 4.7 to 16.8%. As seen in Table 7, there are 12 cases for which the %HbA1c is above the normoglycemic reference range. Six of these twelve are clearly in the range (6.2–6.9) where conclusions about hyperglycemia would be unwise. However, for the six unknowns whose values exceeded 7.0%, the mean was 11.7% which did not differ statistically from the diabetic mean ($p = 0.6615$). Unfortunately, subsequent attempts made to determine if any of these individuals had a medical history of diabetes were unsuccessful. This is not surprising as the Centers for Disease Control estimates that 5.4 million people in the United States have diabetes without the appropriate diagnosis (18). This is further emphasized by two cases which partially prompted these studies. Case 1 involved a 35-year-old male graduate student found dead in his apartment. The case was first suspected to be a drug overdose because of his history of depression. Toxicological studies did not find evidence of any drugs but did find an acetone of 5 mg/dL in urine and 3 mg/dL in blood. When subsequent testing showed an increased %HbA1c (13.1%), the case was investigated further. At this time, friends reported the decedent had noted symptoms consistent with diabetes and had made an appointment with his family physician for evaluation. From this information and the laboratory analysis, the medical examiner ruled that diabetes contributed to this death. Another case involved a 60-year-old female found to have pericarditis and coronary artery disease at autopsy. Again toxicological studies were significant only for an increased acetone (39 mg/dL). Testing showed an increased %HbA1c of 10.9%. Unlike in the previous case, further investigation and interviews found no complaints or suggestion of poor glycemic control or diabetes.

The second case emphasizes the need for additional studies. In the clinical setting, %HbA1c is not considered a diagnostic test for diabetes but a useful tool to assess glycemic control over the past 6–8 weeks. It is important to note that multiple studies have found a linear relationship between the HbA1c and the plasma glucose level. Goldstein et al. found that each 1% change in HbA1c correlated with a 25–35 mg/dL change in average plasma glucose. They also found that regardless of the HbA1c method used, a HbA1c above the reference range by 3% or more was indicative of a mean plasma glucose above 200 mg/dL (19). Nathan et al. found that the HbA1c could be related to the mean capillary, blood glucose measured by the patient at home by the following equation: mean blood glucose $= 33.3$ (HbA1c) $- 86$ (20). Using either of these criteria, the second case would have experienced consistent glucose levels above 200–250 mg/dL during the weeks preceding her death.

One should remember that in the early stages of diabetes, the amount of glycated hemoglobin might be within the normoglycemic range. With progression of the disease, chronic hyperglycemia (defined as glucose $>$ 126 mg/dL) occurs. Numerous studies have shown that this state correlates with the development of microvascular complications. Since maintaining the glycated hemoglobin in the normoglycemic range can minimize these complications, this goal is desirable, especially for type 2 diabetics. Unfortunately, for many type 1 diabetics normalization of the glycated hemoglobin may mean episodes of hypoglycemia, a condition equally hazardous. Thus for these patients, a higher glycated hemoglobin is targeted: those $<$7% suggest excellent control and those in the 7–8.5% suggest good control.

With this in mind, coupled with the finding that some increase in %HbA1c occurs with storage, it is not unreasonable to suggest that when using HbA1c in a postmortem investigation, levels above 8.5% indicate chronic hyperglycemia and those between 6% and 8.4% need further investigation.

Acknowledgment

We would like to thank the staff of the UNC Hospitals McLendon Laboratory and Ms. Gloria Dean for their invaluable assistance in this study.

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