One HbA1c Measurement Does Not Tell the Whole Story – 5 Case Studies

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ABSTRACT

Hemoglobin A1c is produced by an interaction between intracellular glucose and hemoglobin. This is a dynamic process, because glucose values vary and RBCS have a limited lifespan. Hemoglobin A1c testing is performed to monitor glycemic control in patients with diabetes mellitus. The ADA recommends testing HbA1c twice a year in patients with good control, and more often in patients who do not meet their goals. MLS/CLS students are required to have an understanding of the relationship between HbA1c, plasma glucose and time. Case studies are valuable teaching tools to illustrate the dynamic nature of HbA1c formation. This work shows 4 months of serial HbA1c and glucose measurements in 5 individuals with type 2 diabetes mellitus. A stepwise increase or decrease of HbA1c is seen in subjects who have a change in their glycemic control, illustrating why one HbA1c result does not tell the whole story.

ABBREVIATIONS: eAG - estimated average glucose, ADA - American Diabetes Association, HbA1c - Hemoglobin A1c

INDEX TERMS: HbA1c; type 2 diabetes; glucose; diabetes mellitus

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INTRODUCTION

Diabetes mellitus affects 29.1 million people in the United States; 90-95% of these have type 2 diabetes.[1] Patients with diabetes are regular customers of the clinical laboratory. As early as 1997 the American Diabetes Association (ADA) recommended performing a glycated hemoglobin at diagnosis and again every 3-6 months to assess glycemic control for all patients diagnosed with diabetes mellitus.[2] The target goal for HbA1c was established as <7%. This recommendation followed evidence provided by multiple studies that indicated that the risks of chronic complications of diabetes, i.e. cardiovascular disease, retinopathy, neuropathy and nephropathy, can be minimized by keeping blood glucose close to normal.[3,4,5] In 2002 Rohlfing et al. showed a strong correlation between average glucose and HbA1c.[6] In 2008 Nathan and colleagues determined that the relationship of average plasma glucose and HbA1c could be used to estimate average glucose.[7] Their formula is eAG in mg/dL = 28.7 x A1c (in percent) – 46.7. In other words, for an HbA1c of 5%, the estimated average glucose is 97 mg/dL. For each percent HbA1c above 5%, the average glucose increases approximately by 29 mg/dL.[7] Many laboratories include this estimated value with the HbA1c.

In 2010, the workload at the HbA1c bench increased dramatically. That year the ADA endorsed the use of HbA1c for the diagnosis of diabetes mellitus as an alternative to using fasting plasma glucose and glucose tolerance tests, since HbA1c does not require fasting, it is standardized, and it provides information about the average glucose for the previous 2-3 months.[8] The threshold value of $\geq$ 6.5% is used to make the diagnosis. HbA1c (also referred to as glycosylated hemoglobin) continues to be used to monitor glycemic control. The same diagnostic value and target goal for HbA1c still apply in 2015.[9]

An understanding of HbA1c is a requirement for a medical laboratory scientist, and a significant component of the MLS/CLS curriculum. Important considerations for the student are: How does HbA1c

form? What can make the test inaccurate? and Why do we test multiple times per year?

The three components in HbA1c production are glucose, hemoglobin and time. HbA1c is produced by the reversible interaction of intracellular glucose with the N-terminal valine of the beta chains of hemoglobin, eventually forming a permanent bond. Red blood cells (RBCs) are freely permeable to glucose and do not require insulin to facilitate its transport. Therefore, a percent of hemoglobin is glycated in all individuals, those without diabetes (HbA1c reference range <5.7%), and those with type 1 and type 2 diabetes. When plasma glucose values are increased, such as in persons with diabetes mellitus, intracellular glucose also rises. A higher percent of the hemoglobin is glycated. HbA1c can be measured by HPLC, immunoassay or boronate affinity chromatography.

The HbA1c value has a high correlation with average blood glucose for the previous 2-3 months in individuals with either type 1 or type 2 diabetes.[6,7] The look back time period is related to the average time that a hemoglobin molecule is available for glycation. In the peripheral blood, hemoglobin is found in RBCs and reticulocytes. The average life span of RBCs is 120 days.[11] Conditions that alter the life span of the RBC, such as hemolytic anemia, iron deficiency anemia, and blood loss will negate this association.[12] The student should be educated that the presence of hemoglobin variants, such as HbS, HbE and HbC, interferes with some HbA1c methods.[10,13] As yet undetermined variability between individuals can also affect the rate of glycation.[14]

The ADA continues to recommend bi-annual measurement of HbA1c for individuals with diabetes who are in good glycemic control, and more frequent testing if therapy has changed.[10] Blood glucose is influenced by diet, hydration, exercise, illness, and medication.

Case studies can be used as a teaching tool to understand how readily HbA1c can fluctuate with diabetes mellitus, and to evaluate the effect of time in the hemoglobin-glucose dynamic. In this report, 5 individuals were followed for 4 months. One had steady state glucose values that met the target goal; one had elevated values that did not meet the goal. One individual had increasing glucose, and two had decreasing glucose over the study time period. These cases illustrate the dynamic nature of HbA1c formation and the rationale for testing multiple times per year.

Materials and methods

The cases presented here were participating in a study comparing average blood glucose to HbA1c.[15] All subjects had a diagnosis of type 2 diabetes. Subjects met with the researcher at 4 time points during the study, and were aware of their results. Participants tested their own glucose over a 3 month period using a Bayer Contour glucose meter (Daphne, AL) and Ascensia strips. Subjects tested fasting glucose one week out of the month and 2 hour postprandial (lunch or dinner) glucose during a different week out of the month. During the unscheduled weeks, they were instructed to test as they usually would. This was an observational study; no treatment was recommended or provided. Participants were instructed to follow their physician's treatment plan.

HbA1c was performed by immunoassay using a Siemens DCA2000 (Tarrytown, NY). Blood was collected by venipuncture at the initial meeting, and again at 2, 3 and 4 months. Imprecision of the analyzer was determined as a CV of 0.04 at normal HbA1c values and 0.02 at elevated HbA1c values. Anemia and blood loss were ruled out by testing hemoglobin at each collection time (AcT, Beckman Coulter, Fullerton, CA). All participants were negative for HbS, HbE and HbC by Paragon hemoglobin electrophoresis (Beckman Coulter). Participants signed an informed consent. The study was approved by the Institutional Review Board at the University of West Florida.

Five cases demonstrate the dynamics of HbA1c

Steady state

Case 1 was a 56 year old African American male recruited at a church. He stated that he typically performed fingerstick glucose testing 1-2 times per day. He used Metformin, an oral medication, to manage his diabetes. His initial HbA1c was 6.2%, with an estimated average glucose (eAG)[13] of 131 mg/dL. This person showed fairly consistent glucose and HbA1c results during the study, shown in Figure 1A. Fasting glucose measurements had an average of 130 mg/dL, and a standard deviation of 12 mg/dL. The average postprandial glucose was 113 mg/dL with a standard

deviation of 18 mg/dL. The initial HbA1c of 6.2% varied slightly over the course of the study and was 6.6% when measured at four months (Table 1). All results for Case 1 were considered to be within the acceptable target range. eAG for HbA1c of 6.6% is 143 mg/dL.

Case 2 was a 63 year old Caucasian male recruited at a university. He stated that he performed glucose testing before breakfast on most days. He had prescriptions for Metformin, Actos and Glyburide, all of which are oral medication. He shared that he was mistrustful of these medications, and did not always take them. His initial HbA1c was 12.1%, with an eAG of 301 mg/dL. This subject performed testing erratically, so the average fasting and postprandial glucose were not calculated. Capillary glucose results are shown in Figure 1B; results of fasting and postprandial glucose are very high. The fasting glucose measurements he reported ranged from 185 to 371 mg/dL. The postprandial measurements ranged from 215 to 418 mg/dL. HbA1c increased slightly at each time point (Table 1) and the HbA1c was 13.0% at four months, well above the therapeutic goal. eAG for HbA1c of 13.0% is 326 mg/dL. During the course of the study, this subject was hospitalized for several days due to an infected wound. One year after the conclusion of the study, he suffered a myocardial infarction and expired soon afterwards.

Increasing glucose

Case 3 was a 70 year old African American female recruited at a community center. She stated that she performed glucose testing a couple times a week. At the initial meeting she reported that she usually took seven medications but had recently been cut down to three. She could not name the medications, but she did not take insulin. This subject performed fasting glucose measurements almost daily, but neglected to test after meals. Significantly, her fasting glucose and HbA1c steadily increased over the course of the study, beginning at 6.3%, 8.6% at two months, 9.2% at three months, and finally 10.8% at the conclusion of the study. Capillary glucose results over the time period are shown in Figure 2. The average fasting glucose in the fourth month was 369 mg/dL, with a range of 229 to 582 mg/dL. At the end of the fourth month, the researcher met with the participant at her home, where she lived alone. She produced her medications, which were Amlodipine, Omeprazole, and levothyroxine sodium. None of these medications are used to treat diabetes. After being informed of this, the participant visited the county health department, and was prescribed appropriate medication. A follow-up HbA1c done six months later was 6.3%.

Decreasing glucose

Case 4 was a 51 year old Caucasian male recruited at a university. He reported testing his own glucose 2-3 times per week. He joined the study for motivation to improve his glycemic control. His initial HbA1c was 12.4% (eAG of 309 mg/dL) in spite of taking the insulin products Lantus and Humalog along with Metformin. This subject showed a stepwise decrease in HbA1c from 12.4% at the initial visit to 9.6% two months later. By four months, the HbA1c was 8.2%. Average postprandial glucose was 231 mg/dL. eAG for

Table 1. Participant HbA1c and glucose values.

Case ID	1	2	3	4	5
Age	56	63	70	51	53
Gender	Male	Male	Female	Male	Female
Race	African American	Caucasian	African American	Caucasian	Caucasian
Initial HbA1c %	6.2	12.1	6.3	12.4	> 14
2 month HbA1c %	6.9	12.3	8.6	9.6	9.7
3 month HbA1c %	6.8	12.8	9.2	8.8	8.2
4 month HbA1c %	6.6	13.0	10.8	8.2	8.4
Fasting glucose mg/dL*	130 (12)	NA	208 (78)	161 (43)	NA
Postprandial glucose mg/dL*	113 (18)	NA	NA	231 (68)	NA
Initial Hemoglobin g/dL	13.5 g/dL	13.7 g/dL	12.9 g/dL	14.6 g/dL	13.5 g/dL

* Mean (SD); NA: not available

HbA1c of 8.2% is 189 mg/dL.

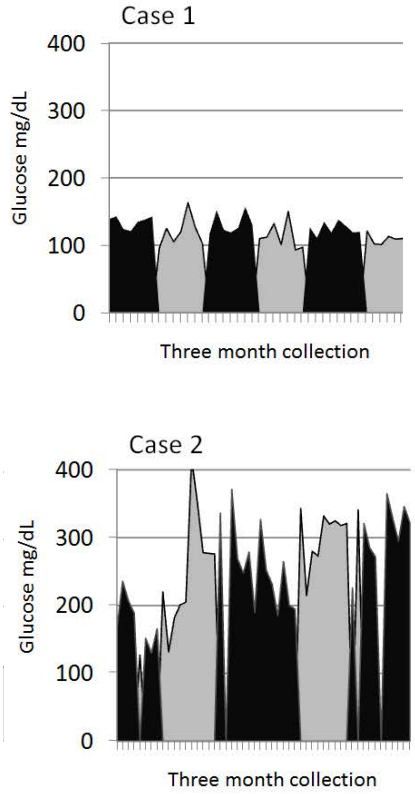

Figure 1. Capillary glucose measurements over three months. Black indicates fasting glucose, gray indicates postprandial glucose. Tick marks indicate individual days that were measured. Case 1 shows glucose measurements that are relatively consistent over the course of the study, with the maximum reading of 163 mg/dL. Case 2 shows inconsistent glucose levels, with several readings exceeding 300 mg/dL. The participant failed to perform many postprandial readings.

Case 5 was a 53 year old Caucasian woman recruited at a university. Her initial HbA1c was over 14%, higher than the linearity of the analyzer (eAG > 355 mg/dL). She was highly motivated to improve her health due to the birth of a grandchild. She was not taking any medication at the beginning of the study. Soon after beginning the study she sought medical advice, she was prescribed Metformin and began therapeutic lifestyle changes. At the two month time point, her HbA1c had decreased to 9.7%. At four months, it was 8.4%. During the study she tested her capillary glucose sporadically; therefore composite data is not available. Estimated average glucose based on 8.4% HbA1c is 194 mg/dL.

Discussion

These case studies help the student gain insight into how HbA1c responds in a stepwise manner to changes in blood glucose. They also add the human component of the equation. HbA1c values are touted as a 3-month composite of average glucose, with the expectation that measurements of the 3 months are similar to each other. This was shown in Case 1 and Case 2. Both of those individuals were educated about their diabetes. Case 1 had been diagnosed 4 years prior to the study. He was able to manage his diabetes by diet, exercise and oral medication. He was meticulous with his recordings for the study. Case 2 was non-compliant with prescribed treatment, and non-compliant with the testing regimen for the study. He had been diagnosed with diabetes nine years prior to the study. He suffered from poor wound healing, and cardiac disease, two complications that are more prevalent in diabetes.

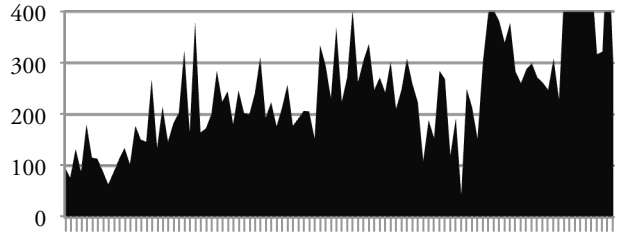

Figure 2. Capillary glucose measurements for Case 3. Fasting glucose measured over 4 months' time. Notice the increasing values, with the final month exceeding 400 mg/dl seven times. The 4 month HbA1c was 10.8%.

Case 3 offers a rare view of increasing HbA1c from normal to high. During the first month of the study, the participant's average fasting glucose was 165 mg/dL, with a range from 64-285 mg/dL. Her HbA1c increased 2.3% in two months' time, and over the 4 months of the study increased 4.5%. Because the glucose was not steady state, the final HbA1c of 10.8% (with its eAG of 263 mg/dL) was misleading. Her most recent average glucose was between 300 and 400 mg/dl (Figure 2). The researcher at first suspected that the participant was noncompliant with her medication, which was in stark contrast with her enthusiasm to participate in this study. Eventually it was discovered that the participant was not actually prescribed medication for her diabetes. It is suspected that the participant had low literacy skills and no support system that could explain or research

her medication regimen. Low health literacy could also explain her lack of follow up when glucose values were obviously high. Unfortunately, this is not uncommon even in educated individuals. In a study of 555 individuals with diabetes, with 87% of participants having an education level of high school graduate or college, 50% of the participants reported that they do nothing when their blood glucose is high. Poor health literacy and poor problem solving skills are a pervasive problem.[16]

Case 4 illustrates the time it takes for HbA1c to decline. The participant was motivated to succeed, and noted that having regular follow-ups with the researcher monitoring his results made a difference to his commitment. His original HbA1c of 12.4% equated to an eAG of 309 mg/dL. His final HbA1c dropped to 8.2%. 8.2% still exceeds the goal of 7.0%, but encouragingly, it is very close to the acceptable value. While this HbA1c value would indicate an average glucose for 2-3 months of 189 mg/dL in a person with steady state glucose, we can see here that some of the preexisting glycated hemoglobin continued to contribute to the higher value. The stepwise decline in HbA1c is associated with the turnover in the RBC population. The older RBCs have had the longest exposure to glucose. As they are removed from the blood, the average red cell HbA1c drops. This stepwise decline is reiterated in Case 5. Case 5 HbA1c decreased between 3-4% in two months, and 6% overall. Interestingly, Case 2, Case 4 and Case 5 were all professionals with post-graduate degrees. Poor literacy skill was not a contributing factor to their elevated initial HbA1c values. Clearly, motivation and trust in medical advice are also important factors to consider in the treatment of individuals with diabetes.

These cases inform the student that one HbA1c does not tell the whole story. HbA1c may increase if the patient is non-compliant with therapeutic lifestyle changes or medication, if he/she is health-illiterate, or if he/she is ill. The patient with a value of 8.8% may be at steady glycemic state, his/her glucose may be increasing or his/her control may actually be improving. One HbA1c value does not allow a full appreciation of the status of the patient. For those patients whose HbA1c is higher than the recommended value, the ADA recommends a repeat HbA1c in three months.[10] Comparing serial HbA1c values will detect the patient whose therapy is not working well, and reinforce the efforts of the patient who has committed to improving his/her glycemic control.

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