



# Assessment of Glycemic Control and Hemoglobinopathy: When HbA1c Testing Is Unreliable in High Risk Cardiovascular Patients

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**Abstract:** To assess the evidence underlying Hemoglobin A1c (HbA1c) in patients with hemoglobinopathy and the use of this modality to evaluate confounders, sources of error, upcoming developments and reach evidence-based conclusions on their optimal use in patients who require tight glucose control. This was a retrospective chart review of 7 patients with data collected between the years 2004 - 2009. Participants were selected based on the criteria that they were 1) diagnosed with Type 2 Diabetes Mellitus; 2) patients of the North Florida Thyroid Center for at least one year; and 3) had hemoglobinopathy. These patients were selected using electronic medical records (EMR). Researchers were blinded of patients' gender, race and any other personal identifiers. A random search was performed for hemoglobinopathy within physician patient database. Assessing glycemia in diabetics can be a challenge, in particular subgroup patients with hemoglobinopathies face several pitfalls that can implicate glycemia assessment rather difficult. Our results suggest there may be a discrepancy between mean blood glucose and A1C levels of individuals with a hemoglobinopathy in small cohorts at risk of cardiovascular demise.

**Keywords:** Cardiovascular, Diabetes, Glycemic, Hemoglobinopathy, HbA1c, Cardiothoracic

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## 1. Introduction

Diabetes is a serious problem with a disease burden of more than 23.6 million Americans [8]. "Diabetes is a disease in which the body does not produce or properly use insulin" [8]. There are several types of tests that are used today to test for diabetes. One test is the hemoglobin A1c test which measures the percentage of glucose that binds to hemoglobin, usually over a three-month period [7]. Analysis of glycated hemoglobin (HbA1c) in blood provides evidence about an individual's average blood glucose levels during the previous two to three month [11]. Other tests can be used to self monitor the daily blood glucose levels for individuals diagnosed with diabetes, which shows the immediate blood glucose levels [9].

Diabetes is particularly a harmful epidemic because of its effects on the cardiovascular system. Diabetes has been known to lower good cholesterol influencing the proportion of atherosclerotic plaques of small vessels throughout the body. Studies have shown diabetes to be as a strong risk factor for cardiovascular health as having a myocardial

infarction and a likelihood of having future increase risk of morbidities and mortalities following surgery [12]. The glycosylated hemoglobin test also known as the HbA1c test is administered by many practitioners to provide a picture of the previous months of glucose levels. Adequate monitoring helps in providing the intensive and strict care that patients who are at risk of cardiovascular disease may require.

The Diabetes Control and Complication Trial of 1993 was designed to determine if intensive treatment could decrease the frequency and severity of microvascular and neurologic complications [3]. This study showed that keeping blood glucose levels normal slowed the progression of diseases of the eye, kidney, and nervous system. During the trial they performed a hemoglobin A1C test to estimate the average blood glucose for these individuals. The researchers believed that A1C is one of the best tests to estimate an individual's mean blood glucose [3].

In patients with diabetes and normal hemoglobin levels, A1c values strongly correlate with blood glucose levels [10]. However diabetic patients with an abnormal hemoglobin may

produce unreliable A1c results. This is due to a decreased percentage of glycated hemoglobin and decreased erythrocyte survival. These characteristics can dramatically affect A1c test results by altering the normal process of glycation of hemoglobin to A1c [10]. Because of this discrepancy the purpose of our research is to examine the relationship between A1c levels and mean blood glucose in diabetics with hemoglobinopathies. We hypothesize that there will be a difference in the correlation between mean blood glucose and A1c in diabetic patients with abnormal hemoglobin compared to diabetic patients with normal hemoglobin.

Hemoglobinopathy is a genetic and heritable trait in which abnormality exists in one or more of the globin chains in the hemoglobin [5]. Common hemoglobinopathy diseases are thalassemia, sickle cell anemia, hemoglobin C disease, and hemoglobin SC disease. Two-tier hemoglobin electrophoresis cellulose acetate electrophoresis and thin-layer isoelectric focusing are widely used screening tests for hemoglobin disorders. Electrophoresis is highly specific in the detection of certain hemoglobin disorders, especially sickle cell disease. The hemoglobin variance types are listed in Table 1.

According to a study by Adekanmbi et al. it was found that patients with elevated HbF, have a falsely low HbA1c [1]. This may result in missed diabetes diagnoses and the under treatment of diabetics [1]. On reproduction of non HbA studies, immunoassay-based methods such as those using antibodies produced results that were falsely low. Furthermore, high performance liquid chromatography (HPLC) and electrophoresis could not produce reliable results.

*Table 1. Common Hemoglobinopathies.*

Hemoglobin (Hb) Variant
Hemoglobin S (HbS)
Hemoglobin C (HbC)
Hemoglobin E (HbE)
Hemoglobin SC (HbSC)
Hemoglobin F (HbF) elevated

## 2. Methods

### 2.1. Selection Criteria

7 participants number 127-133 were selected based on the criteria that they were 1) diagnosed with Type 2 Diabetes Mellitus; 2) patients of the North Florida Thyroid Center for at least one year; and 3) had hemoglobinopathy. These patients were selected using electronic medical records (EMR). Researchers were blinded of patients' gender, race and any other personal identifiers. Physician performed a random search for hemoglobinopathy within physician patient database.

### 2.2. Research Design

This was a retrospective chart review of patient data

collected between the years 2004 - 2009. Data collection included the downloading of each patients' blood glucose meter readings to a computer and electronic storing of data using EMR. The meters used by patients were selected at patient discretion. The blood glucose readings were downloaded at each office visit. Quarterly, the endocrinologist administered a hemoglobin A1c test to each patient and obtained the results from a laboratory. Patients' blood was drawn and sent to a laboratory for hemoglobin analysis to determine hemoglobin A1c levels. All lab test for A1c were determined using electrophoresis. The laboratory results from each patient were used to record the hemoglobin A1c.

Average blood glucose and HbA1c were compared to determine which results were taken during the same time period. In order to be included in the analysis, the blood glucose reading download and A1c results had to have been recorded within 30 days of the other. The raw data was then compiled into Excel spreadsheets to identify trends. All data was analyzed using the Statistical Package for Social Sciences (SPSS) for Linear Regression Model, DCCT data comparison and Characteristic Table.

## 3. Results

Expected blood sugar levels based on A1c and observed mean blood sugar levels vary. Some patients had relatively high mean blood glucose compared to their A1C. For patient 128, their average blood glucose was 119 and A1C was 8.9. In patient 125, their average blood glucose was 201 but A1C was 6.9. This indicates that there may be a discrepancy in the A1C test. Please see Figure 1 for a graphical depiction of the discrepancy of the A1C test having wide variation in mean blood glucose levels to corresponding A1C. Table 2 shows the patients average blood glucose reading and their corresponding A1C levels.

*Table 2. Mean blood glucose and A1C of patients 127-133.*

Patient ID	Mean Glucose	A1C
128	119	8.9
128	105	6.2
128	102	6.1
128	104	6.4
128	97	6.2
133	125	6.9
133	143	7.4
132	139	5.7
129	185	8.2
129	223	8.2
127	106	7.5
131	203	10.9
131	223	8.8
131	171	7.2
130	201	7.2
130	207	8.5

Several patients' actual mean blood glucose levels were significantly different from that of the estimated mean blood

glucose when utilizing the formula. The standard deviation for mean blood glucose readings is  $\pm 15.7$ . Note that for most patient observations, the difference between actual and estimated blood glucose levels was greater than that of the

standard deviation. These results provide more evidence that the A1C test may be less accurate when testing individuals for HbA1c who have concurrent hemoglobinopathy.

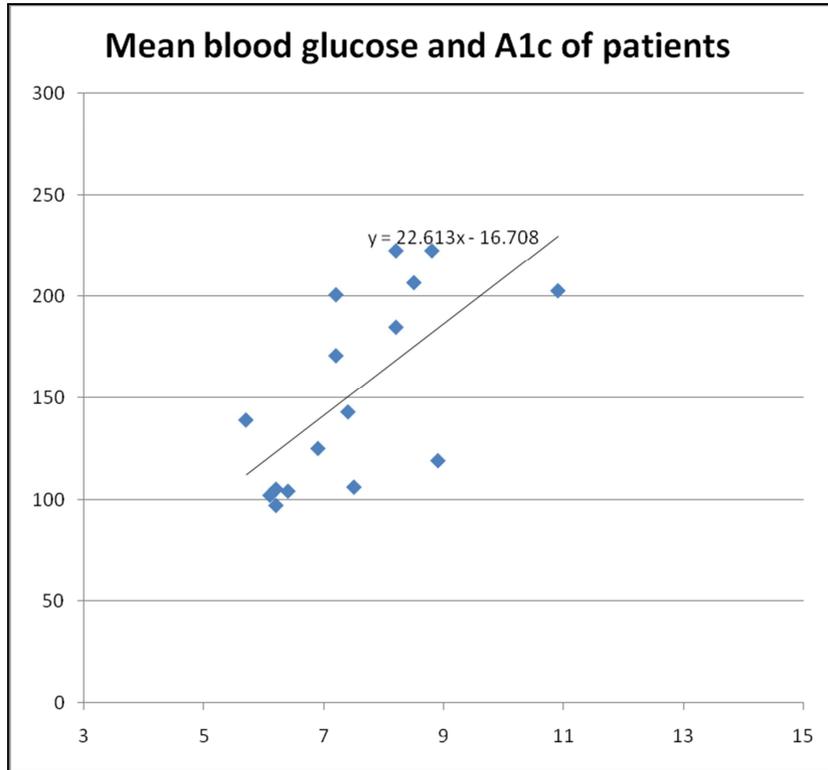


Figure 1. HbA1c and Mean Blood Glucose.

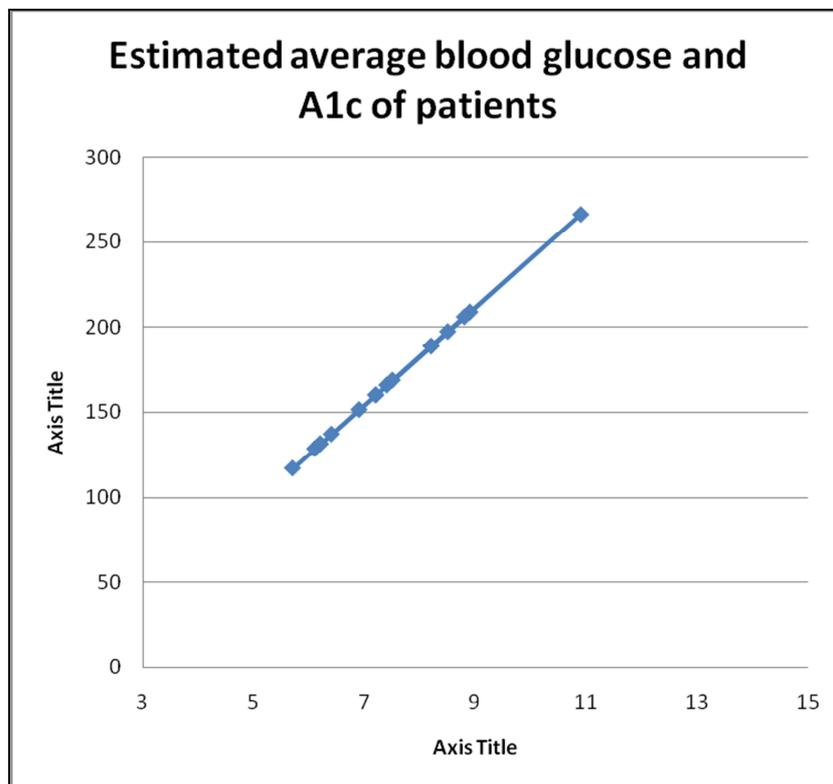


Figure 2. Estimated Blood Glucose per HbA1c.

**Table 3.** The difference between actual mean blood glucose and estimated blood glucose.

Patient ID	Mean Glucose	Estimate Glucose	Difference
128	119	208.73	89.73
128	105	131.24	26.24
128	102	128.37	26.37
128	104	136.98	32.98
128	97	131.24	34.24
133	125	151.33	26.33
133	143	165.68	22.68
132	139	116.89	-22.11
129	185	188.64	3.64
129	223	188.64	-34.36
127	106	168.55	62.55
131	203	266.13	63.13
131	223	205.86	-17.14
131	171	159.94	-11.06
130	201	159.94	-41.06
130	207	197.25	-9.75

## 4. Discussion

Our results illustrated that there is a discrepancy between mean blood glucose and A1c levels of individuals with a hemoglobinopathy. The Diabetes Control and Complications Trial made A1c the test of choice for diabetes due to its ease in determining glucose levels over a period of time [13]. There are several types of common hemoglobinopathies which can result in inaccurate test results for these particular individuals. (Table 1) According, to the National Diabetes Information Clearinghouse, “African Americans have an increased risk of inheriting sickle cell trait, the condition in which people have both hemoglobin A (HbA), the usual form of hemoglobin, and hemoglobin S (HbS), a variant. They also are at risk for having hemoglobin C (HbC), another variant. According to Behan et al. “Hemoglobin variants may invalidate HbA1c results in vitro by analytical interference or in vivo by altering RBC lifespan or glycation rate” [2].

Hemoglobin is a protein that has the ability to carry oxygen in the blood. Less than 10 percent of the Hemoglobin protein is found in blood, bound to glucose; Hemoglobin A1C. Hemoglobin A is the most common of the hemoglobins without abnormality. Hemoglobin S – Most common form of abnormal hemoglobin and the basis for sickle cell trait and anemia. There is a change in the 6<sup>th</sup> Amino Acid where glutamine should be. Hemoglobin C- This hemoglobinopathy is due to an abnormal hemoglobin substitution of lysine for glutamic acid at the 6th position of the  $\beta$ -globin chain. The normal shape of the cells is greatly affected however this disease has proven to be affective against Malaria. Hemoglobin C is more commonly found in West Africa. Hemoglobin SC- Due to the fact that nearly ten percent of African Americans have Hemoglobin S, the Heterozygous Hemoglobin S-C is more popular than Homozygous Hemoglobin C. Milder symptoms sickle cell plagues those diseased with this hemoglobinopathy. Hemoglobin E- Common in South East Asia Hemoglobin E is the second

most popular hemoglobinopathy, after Hemoglobin S. Caused by a substitution in the 26 amino acid.

Sickle cell trait is present in approximately 8% of black Americans and as high as 20% of some African populations [6]. In regards to diabetes in 2007 7.8% of Americans were diagnosed with diabetes accounting for 17.9 and 5.6 known and unknown cases consecutively for a total of 23.6 million individuals. [8]. Therefore, one can deduce that many African Americans and Americans alone can both diabetes and sickle cell trait with sickle cell trait being one of many errors suffered to the hemoglobin protein. Therefore, having a standardized test which will not account for such individuals that have this hemoglobinopathy is detrimental to the health of such individuals. “A1C tests in patients with hemoglobinopathies result in falsely high outcomes, overestimating actual average blood glucose levels for the previous 2 to 3 months.” [4]. Inaccurate test results can also be due to alcoholism, lead poisoning, opiate addiction, excessive use of salicylate, and pregnancy [13]. There are other tests such as the fructosamine test but due to the lack of further research it has not been certified as the official test for individuals with diabetes.

In a case series by Tran et al. a 55-year-old man of Southeast Asia and a 60-year-old obese and hypertensive woman serve as constant reminders of the inaccuracies of the HbA1c on a hemoglobin chromatogram as the Asian man’s Hemoglobin E misrepresented the HbA1c curve and the 60-year-old obese woman had an abnormal Hb peak, confirmed to be known as Hb British Columbia [13]. HbA1c serves as a great marker for diabetic control but is subject to interference in its presence of associated comorbidities such as hemoglobinopathies, hemolysis, renal failure and alcoholism.

Our research showed a disparity between A1c and mean blood glucose we had several limitations. Limitations such as a low sample size can skew the results and not measuring different of hemoglobinopathies can give an inaccurate portrayal of A1C versus mean blood glucose. An explanation of the disparity could be due to the small sample. Further research needs to be done with a larger sample size.

### 4.1. Limitations of Research

Some limitations of research were that the data that we analyzed had limited analysis because of the small sample pool. This sample pool size prevented the generalization of the overall population and its correlation to it being randomized observed patients. This sample pool was further limited by the institution at which the results were found. To adequately analyze the sample, we found it very important that the patients would not be analyzed as a linear regression individually. This was in part of the amount of mean blood glucose meter results paired with the A1c test results. Another limitation in our experiment was due to the number of glucose meter readings. The glucose meter readings were limited in number and varied among different patients. Patients with limited individual data points tend to have diminished, natural tendencies of fluctuations as well as

diminished patterns in hyperglycemia to show. With large data sets individuals and allowed little to Discrepancies and limitations can arise when the individual points are not an accurate value of the time span. With a larger data set as well larger sample size technical data software could be used to help interpret results. Since the participants in the study were middle age adults without active comorbidities in great health, their results could not be generalizable to individuals with comorbidities or non-middle aged adults.

#### 4.2. Why This Research Important

This research is becoming increasingly significant because of the ever-increasing occurrence of diabetes. The successful administration of blood glucose control is increasingly of importance. Many practices control blood glucose levels of their patients with the surveillance of the HbA1C. We describe the discrepancies and pitfalls that can arise and thus must be considered in the overall observation of various patients. Our research hopes to be the cause for further research and the creation of another test or education for physicians to acknowledge the inaccuracies of the A1c test.

#### 4.3. Areas of Further Research

In regards to further research a larger sample size looking at other centers would afford more power in analysis of data. Studies with minorities prove difficult as their proportion in studies may be lower. Using comparisons of Caucasian individuals with abnormal hemoglobin provides a discrepancy to the A1C and is thus contraindicated. Caucasians and others with this cohort provide discrepancy to the populations at large. Determining alternative testing for individuals with abnormal Hb is another area of research.

## 5. Conclusions

Our results suggest there may be a discrepancy between mean blood glucose and A1C levels of individuals with a hemoglobinopathy. The Diabetes Control and Complications Trial made A1C the test of choice for diabetes due to its ease in determining glucose levels over a period of time [13]. There are several types of common hemoglobinopathies which can result in inaccurate test results for these particular individuals. (Table 1)

Although our research suggests disparities between A1C and mean blood glucose we had several complications due to limitations. Limitations such as a low sample size can skew the results; measuring few hemoglobinopathies could give an inaccurate portrayal of A1C versus mean blood glucose. This sample pool size prevented the generalization of the overall population.

Our research hopes to be the cause for further research that include a larger sample size, Caucasians with abnormal hemoglobin, and a wider range of minorities. Attention to this topic will lead to the creation of another test or more

education for physicians and patients to acknowledge the inaccuracies of the A1C test. This research is essential for the health of millions with and without diabetes because of its increasing occurrence. Accurate testing of blood glucose is needed to ensure that there are not any discrepancies.

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