

# Fourth Reported Case of Hemoglobin South Florida: A Rare Hemoglobin Variant That Causes Falsely Elevated HbA1c Values

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## Abstract

We report a case of a rare hemoglobin (Hb) variant, Hb South Florida (Hb-SF) with only three cases reported worldwide to our knowledge since initial description in 1985. It causes falsely elevated glycosylated hemoglobin (HbA1c) results during standard laboratory testing in the range of poorly controlled diabetes mellitus. A 42-year-old woman with a history of sclerosing bone dysplasia was noted to have an elevated HbA1c (13.8%) during routine blood work using the ion-exchange high performance liquid chromatography (HPLC) method. The patient did not have a prior diagnosis of diabetes mellitus and denied any symptoms consistent with diabetes. Physical exam was unremarkable. Her blood sugars were normal to minimally elevated. Over several months, she was noted to have persistent elevation in HbA1c in the range of 12-14%. She remained asymptomatic. The patient was referred to endocrinology clinic. Hb electrophoresis was performed due to suspicion for a hemoglobinopathy. The patient was noted to be heterozygous for a rare Hb variant called Hb South Florida that causes falsely elevated HbA1c levels. Her corrected HbA1c, when evaluated by affinity column HPLC, was in normoglycemic range. Hb South Florida was first described in 1985 in Tampa Florida and has since been reported in two other cases worldwide. The Hb is not associated with any clinical disorder reported in literature. There are many commercially available tests for measuring HbA1c levels. Additionally, structural variants in Hb interfere with many of the methods. Ion-exchange HPLC separates Hb based on their charge differences. Hb South Florida variant substitutes a neutral amino acid for a charged one, with valine substituted by methionine. This alteration decreases the transit time in the ion exchange column and causes it to co-elute together with HbA1c, giving a falsely elevated value as they get measured as same species. Affinity chromatography is based on affinity of a species for a particular ligand. It recognizes the structure of the N-terminal glycosylated amino acids of the beta chain of Hb. In the case of Hb South Florida, it was able to separate the variant Hb from glycosylated species. This is the fourth case of Hb variant South

Florida to our knowledge. It is important for clinicians to keep a wide perspective when making an assessment of diabetes solely based on HbA1c values. Patients who do not present with symptoms consistent with diabetes, and have normal blood glucose should prompt a further workup for abnormal Hb variants. This should be done in order to prevent incorrect diagnosis and treatment.

**Keywords:** Hemoglobinopathy; South Florida; Diabetes mellitus

## Introduction

Measurement of glycosylated hemoglobin (HbA1c) is an important test in management of patients with diabetes to assess glycemic control [1]. We report a fourth case of a rare hemoglobin (Hb) variant, Hb South Florida (Hb-SF) with only three cases reported previously worldwide to our knowledge since its initial description in 1985. It causes falsely elevated HbA1c results during standard laboratory testing in the range of poorly controlled diabetes mellitus [2].

## Case Report

A 42-year-old female with a history of sclerosing bone dysplasia was found to have an elevated HbA1c of 13.8% during routine lab work using the ion-exchange high performance liquid chromatography (HPLC) method. Repeat testing showed HbA1c of 12.8% with fasting blood sugar of 98 mg/dL. Patient did not have a prior diagnosis of diabetes mellitus and denied any symptoms of polyuria, polyphagia, or polydipsia. She denied any family history of diabetes. Physical exam was unremarkable. Routine lab work that included a complete blood count, lipid and renal panel was within normal limits. Her home medication included acetaminophen/codeine that she takes for bone pain from sclerosing bone dysplasia. She was asked to repeat lab work over a course of several months, which showed persistent elevated HbA1c in the range of 12-14% with mildly elevated blood sugar readings (Table 1).

Patient remained asymptomatic. Her random blood glucose readings did not correspond to HbA1c values. She was referred to the endocrinology clinic for further evaluation. Hb

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**Table 1.** HbA1c Results

Date	HbA1c results done by ion exchange HPLC	Blood glucose (mg/dL)	HbA1c done by affinity column HPLC
September 13, 2013	13.8%	98	
September 17, 2013	12.7%		
October 02, 2013	12.8%	103	
April 05, 2014	14.1%	109	
April 16, 2014	12.7%	133 (non-fasting)	4.2%

electrophoresis was performed due to suspicion for a hemoglobinopathy. The results showed that the patient was heterozygous for a rare Hb variant called Hb-SF that causes falsely elevated HbA1c levels [2]. Her corrected HbA1c, when evaluated by affinity column HPLC showed results in the normoglycemic range (Table 2).

## Discussion

Glycosylation is the addition of glucose molecules to amino groups of proteins in a non-enzymatic way [3]. HbA1c is the most abundant fraction of glycosylated hemoglobin, proportional to average blood glucose concentration over the previous 2 - 3 months [1]. The concept of measuring HbA1c is based on the fact that Hb formed in new red blood cells enters the circulation with a minimal glucose attached [4]. Red blood cells are freely permeable to glucose and Hb becomes irreversibly bound with it at a rate dependent on the surrounding blood glucose concentration [4].

Healthy adults Hb consists of approximately 97% HbA, 2.5% HbA2 and 0.5% HbF. Hb A is composed of four polypeptide chains: two alpha and two beta chains held together by non-covalent bonds [5]. Glycosylation occurs by a non-enzymatic reaction between glucose and NH<sub>2</sub> groups of the N-terminal valine of the beta globin chains [4]. This is a two-step process. First a reversible reaction between the free aldehyde group of glucose and a free amino group on the Hb molecule forms a Schiff base [6]. This is followed by an irreversible Amadori rearrangement, where the Schiff base is converted to 1-deoxyfructose, producing glycosylated hemoglobin [6].

There are many commercially available tests for meas-

uring HbA1c levels. Methods of identification are based on either physical, chemical or antibody characteristics [5]. Additionally, structural variants in Hb interfere with many of these methods [7]. There have been more than 700 Hb variants reported which arise from point mutations in one of the four chains [5]. The current estimation of Hb variants worldwide is thought to be around 7% of the population [3].

The four most common types of methods of measuring HbA1c are affinity column immunoassay, ion-exchange HPLC, boronate affinity HPLC and enzymatic assay [3]. In the affinity column immunoassay method ligands recognize the structure of the N-terminal glycosylated amino acids of the beta chain. Ion-exchange HPLC separates Hb based on their charge differences [5]. Boronate method uses acid, which reacts with groups of glucose, bound to Hb. Enzyme-linked method uses an enzyme, which reacts with the N-terminal valine of the Hb [3].

As mentioned previously, ion-exchange chromatography is a commonly used method to separate Hb chains, as they elute in the exchange column at different times based on their charge differences [5]. Variant Hb that elute separately from HbA1c are not included in this measurement. Abnormal results occur when a Hb variant cannot be separated from the HbA1c fraction [5]. Hb-SF variant substitutes a neutral amino acid for a charged one, with valine substituted by methionine [8]. This alteration decreases its transit time in the exchange column and causes it to co-elute together with HbA1c, giving a falsely elevated value as they get measured as the same species [5].

There are many common hemoglobinopathies such as sickle cell disease, which are known to cause abnormal HbA1c results. These variants can cause either increased or decreased HbA1c levels, depending on the test type used [7].

Hb-SF was first described in 1985 in Tampa Florida in a 9-year-old boy, referred to the South Florida Diabetes Center. He presented with symptoms of poor weight gain, polyuria, a normal glucose tolerance test, a normal Hb electrophoresis (cellulose acetate method) and HbA1c of 14.8% (ion-exchange chromatography) [2, 9]. Despite the normal electrophoresis results, there was a suspicion for an Hb variant due to elevated HbA1c levels in the absence of diabetes. The authors found that the ion-exchange chromatography results were not consistent when repeated with affinity column immunoassay testing. The latter was able to correctly report the patient's HbA1c, without the false values caused by the non-glycosylated hemoglobin. They concluded that when this Hb variant is suspected the test should be repeated with affinity

**Table 2.** Hemoglobin Electrophoresis Results: April 21, 2014

Hb type	Patient results %	Normal range
Hb A	58.1	> 95%
Hb F	1	Ref: none
Hb S	0	Ref: none
Hb C	0	Ref: ≥ 0
Hb A2	3.7	Normal: 0.7 - 3.1
Hb variant South Florida*	37.2	

\*Pattern consistent with heterozygous Hb South Florida.

column testing [2, 9]. The variant Hb-SF has since been reported in two other cases worldwide, one case in Turkey in 2013 [10] and one in Malaysia in 2006 [11]. The variant Hb is not associated with any clinical disorder reported in literature [9].

In the case of our patient, she initially underwent HbA1c testing with ion-exchange chromatography that showed elevated levels. This was subsequently repeated with affinity chromatography, which showed normoglycemic results analogous to the original case from Florida.

### Conclusion

This is the fourth case of Hb-SF to our knowledge. It is important for clinicians to keep a wide perspective when making an assessment of diabetes solely based on HbA1c values. Patients who do not present with symptoms consistent with diabetes, and have normal blood glucose should prompt a further workup for abnormal Hb variants. This should be done in order to prevent incorrect diagnosis and treatment. It is also important to remember that there are many Hb variants in the populations, some of which are clinically silent but interfere with routine laboratory tests.

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### Conflict of Interest

The authors of this article have no conflict of interest to declare.

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