

# Very Low HbA1c Values in A Patient with Clinical Silent Hemoglobin Variant ( Hemoglobin J ) — A Case Report

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## Abstract

Glycohemoglobin (GHb) most accurately reflects the previous two to three months of glycemic control. According to the American Diabetes Association, GHb should be measured regularly in all patients with diabetes, and values should be maintained below 7% to prevent the risk of chronic complications. Herein, we report a case of a 71-year-old type 2 diabetic female patient with extraordinary low HbA1c values. The hemoglobin electrophoresis identified the existence of Hemoglobin (Hb) variant - Hb J. Our case study emphasized that clinical laboratories should be aware of limitations of their HbA1c assay methods as well as the importance of visual inspection of ion exchange chromatograms to detect abnormalities caused by Hb variants. ( J Intern Med Taiwan 2007; 18: 45-50 )

**Key Words** : Glycohemoglobin, Diabetes mellitus, Hemoglobin variant, Hemoglobin J

## Introduction

Glycohemoglobin (GHb), measured as HbA1c, is an invaluable tool for monitoring long-term

glycemic control, and as such it is a key issue in diabetes care. According to the American Diabetes Association, GHb should be measured regularly in all patients with diabetes, and values should be main-

tained below 7% to prevent the risk of chronic complications<sup>1</sup>. Glycated hemoglobin (Hb) is the result of irreversible nonenzymatic glycation at one or both N-terminal valines of the Hb  $\beta$  chain<sup>2</sup>. This irreversible non-enzymatic reaction between glucose and Hb A, the main type of Hb in normal adults, occurs during the life span of the erythrocyte. The total amount depends on the average glucose concentrations in two to three months before the measurement<sup>3</sup>.

Although several methods based on different principles (high performance liquid chromatography (HPLC), immunoagglutination, boronate affinity assays, and electrophoresis) have been developed, the designated DCCT comparison method is a cation exchange HPLC (Diamat; Bio-Rad, Richmond, California, USA)<sup>4</sup>. In addition, structural variants and chemical derivatives of Hb may interfere with many methods. In such populations with Hb variants, misleadingly high or low GHb values have been identified by some methods, but not by the others. Among these, the influence is thought to be greater when ion exchange HPLC is used<sup>5,6</sup>.

More than 700 characterized Hb variants have been reported<sup>7</sup>. Most mutations in the globin genes of Hb are a single base-pair change in the DNA code resulting in an amino acid substitution<sup>7</sup>. The majority arise from point mutations in the  $\alpha$ ,  $\beta$ ,  $\gamma$ , or  $\delta$  Hb chains<sup>7</sup>. The widespread measurement of GHb has identified new variants, many of which produce no phenotypic abnormalities<sup>7</sup>.

Herein, we report one diabetic patient presented with a very low value of HbA1c. The Hb electrophoresis identified the presence of the Hb variant – Hb J.

## Case Report

A 71-year-old female, who had had type 2 diabetes for about 23 years, was receiving regular treatment in the outpatient department (OPD) of Division of Endocrinology and Metabolism in Kaohsiung Veterans General hospital for many years. The oral

antidiabetic drugs currently being taken by the patient included Repaglinide, Metformin and Rosiglitazone. However, her fasting sugar was not constantly maintained, with the values ranging from 142 mg/dl to 304 mg/dl. In addition, the HbA1c values were not comparable and fluctuated from 3.2% to 7.4%. After loss of follow-up for three months, she visited the OPD again. Low fasting sugar (74 mg/dl) combined with a surprisingly low HbA1c value (0.8%) were found. The renal function was normal (serum creatinine 1.3 mg/dl). The patient was not anemic (Hb 14.1 g/dl), nor was she alcoholic.

HbA1c values were measured by the ion-exchange HPLC methods (HLC-723 GHb V A1c 2.2, Tosoh Corporation, Tokyo, Japan) in the Division of Endocrinology and Metabolism of Kaohsiung Veterans General hospital. As can be seen in the standard GHb report by ion exchange chromatograms

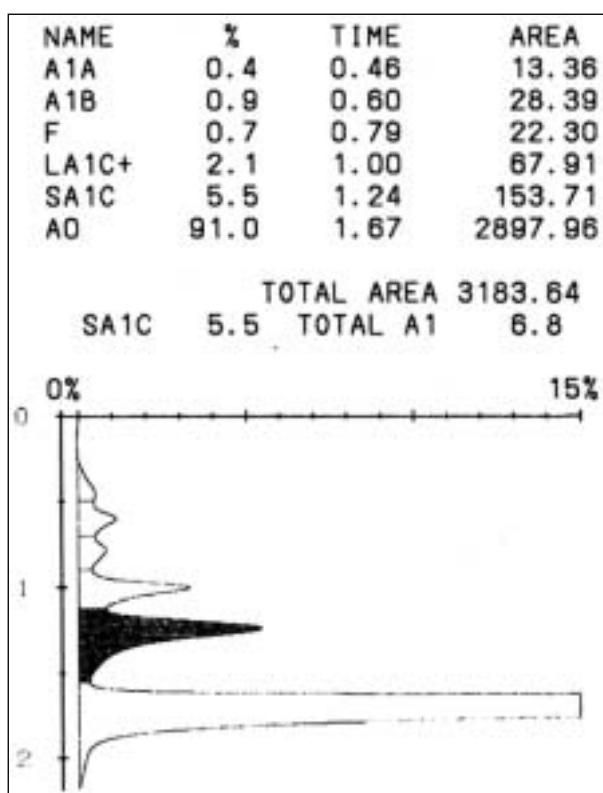


Fig.1. The standard ion exchange chromatogram detected by HLC-723 GHb V A1c 2.2. There were several peaks including A1a, A1b, F, Labile (L) A1c, Stable (S) A1c, and A0. HbA1c is expressed as the percentage of SA1c among total Hb.

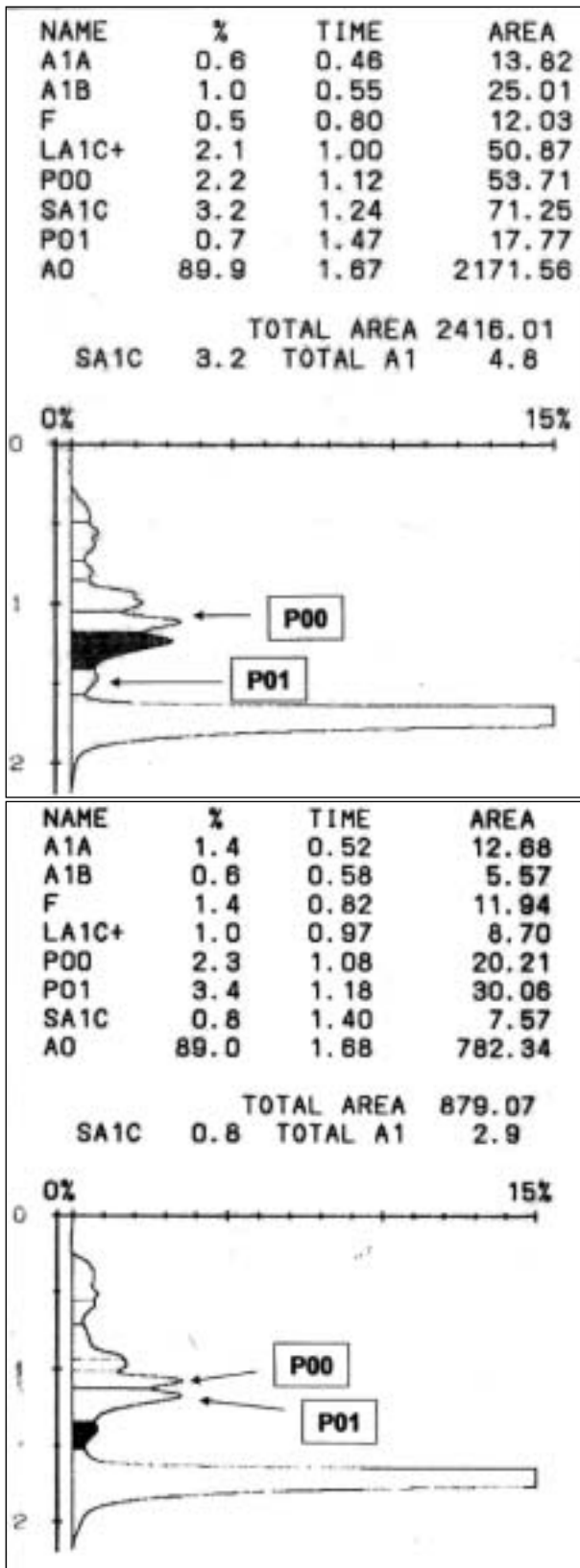


Fig.2. The patient's ion exchange chromatograms detected by HLC-723 GHb V A1c 2.2. Additional peaks (P00 and P01) were found in the chromatogram. A. HbA1c 3.2%; B. HbA1c 0.8%.

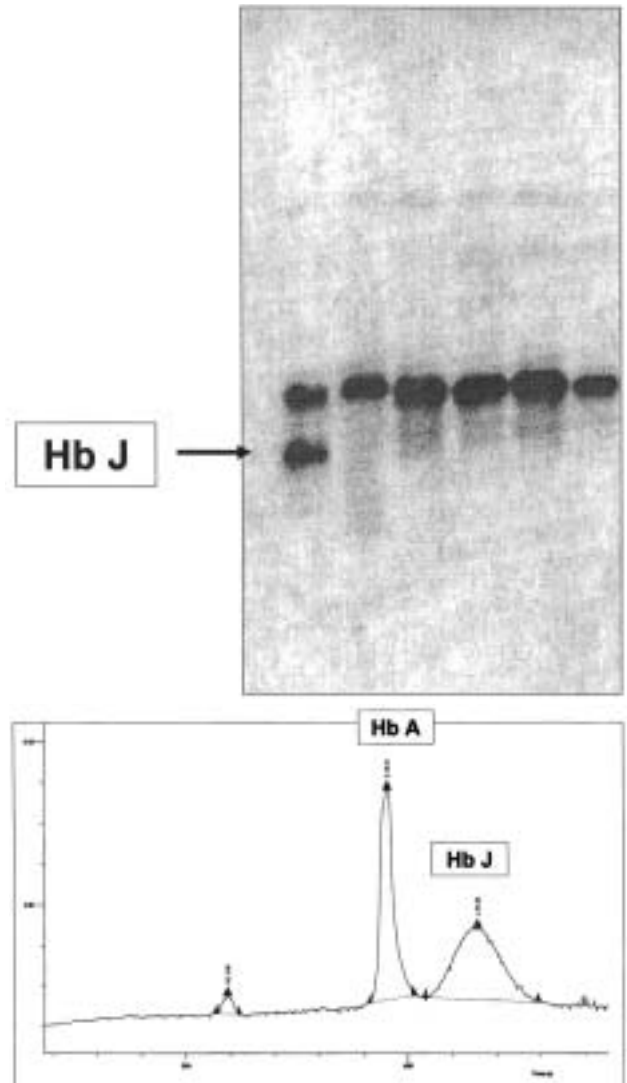


Fig.3. The hemoglobin J, one of the hemoglobin variants, was identified by A. cellulose electrophoresis; B. capillary electrophoresis.

shown in figure 1, there were several peaks including HbA1a, HbA1b, HbF, Labile (L) HbA1c, Stable (S) HbA1c, and HbA0. HbA1c is expressed as the percentage of stable HbA1c among total Hb. In a normal healthy subject, the separation of each peak should be clear. In the appearance of extraordinary peaks, the non-separation of each peak, or the HbF larger than 2%, the values of HbA1c can be misestimated. Hemoglobinopathy should be considered for these patients. After inspecting the ion exchange chromatograms of the patient's GHb report, we found the occurrence of additional peaks (P00 and P01) (Fig. 2). Therefore, Hb electrophoresis was per-

formed to detect the existence of Hb variant. As we predicted, a Hb variant – Hb J was identified by cellulose and capillary electrophoresis (Fig. 3).

## Discussion

In normal adults, Hb consists of ~ 97% HbA, 2.5% HbA2, and 0.5% HbF<sup>8</sup>. Fractionation of HbA by chromatography identifies several minor peaks referred to as HbA1, or fast Hbs, which include the glycosylated forms HbA1a, HbA1b, and HbA1c<sup>8</sup>. These fast Hbs form as the result of a two-step reaction. In the first step, a reversible reaction between the free aldehyde group of glucose or other sugars and non-protonated free amino groups on the Hb molecule forms a Schiff base. This reversible reaction is followed by an irreversible, nonenzymatic Amadori rearrangement that produces GHb<sup>8</sup>. The glycation alters the structure of the Hb molecule and decreases its net positive charge. Many forms of testing use one or both differences to separate GHb from nonglycosylated Hbs.

The N-terminal valine of the  $\beta$  chain provides the most common site of glycation within the Hb tetramer, accounting for 80% of HbA1<sup>8</sup>. The International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) defines HbA1c as Hb that is irreversibly glycosylated at one or both N terminal valines of the  $\beta$  chains. The remaining GHbs contain glucose, glucose-6-phosphate, fructose-1,6-diphosphate, or pyruvic acid bound to 1 of 44 additional sites occurring at  $\epsilon$ -amino groups of lysine residues or at the NH<sub>2</sub> terminus of the  $\alpha$  chain<sup>8</sup>. Although all commercially available methods include HbA1c in GHb measurements, they vary in their ability to detect non-A1c GHb.

Depending on the determination method, the concentration of HbA1c is approximately 4-6% in healthy patients without diabetes. Glycosylated Hb most accurately reflects the previous two to three months of glycemic control. In clinical practice, the measurement of HbA1c is required every three months to

determine whether the patient's metabolic control has remained steady within the target range.

However, the presence of Hb variants may falsely produce low values for HbA1c or spuriously increased HbA1c values. The identification of Hb variants is, therefore, important to avoid inaccurate GHb results. In addition, several other factors besides the presence of genetic variants or chemically modified derivatives of Hb<sup>6</sup>, such as drugs, anemia, uremia, and alcoholism, may falsely lower GHb results. Decreased red blood cell survival and mean erythrocyte age falsely lower GHb values<sup>6</sup>. Blood loss, hemolytic anemia, sickle cell anemia, and chronic renal disease affect the life span of red blood cells and are known to be associated with underestimated GHb values<sup>5</sup>. In contrast, iron and B12 vitamin deficiency have been reported to overestimate GHb results<sup>9</sup>.

The National Glycohemoglobin Standardization Program (NGSP) evaluates and certifies methods on the basis of specific precision and bias criteria. However, samples with variant Hbs and other known interferences are excluded from certification testing, and there are no specific guidelines for comparability of results from samples containing Hb variants. Nevertheless, interference caused by Hb variants needs to be evaluated for each HbA1c method.

There are more than 30 different GHb assay methods available, but the majority of HbA1c measurements are performed by ion-exchange HPLC or immunoassay. An advantage of most ion exchange HPLC methods is that they allow detection of most Hb variants present<sup>10</sup>. In contrast, the enzymatic method, boronate affinity and immunoassay methods do not allow recognition of a variant Hb.

In general, a Hb variant can be suspected in patients with HbA1c results >15%, HbA1c measurements below the nondiabetic reference range, or when the HbA1c result varies substantially from other indices of metabolic control and/or clinical impression.

With the ion-exchange HPLC methods, clinical silent Hb variants may cause additional peaks in chro-

matograms resulting in false or no HbA1c value. Late migrating Hb variants may not be detected in a chromatogram, as demonstrated with the Tosoh ion exchange HPLC method HLC-723, but could still interfere to cause falsely low HbA1c results.

The influence of Hb variants on GHb determination is great when using ion exchange HPLC. Therefore, if an erroneous result is caused by Hb variants, affinity chromatography may provide a more accurate measure of HbA1c. Furthermore, fructosamine could be used as a comparison because nonenzymatic glycation of serum proteins, mainly albumin, should not be influenced by Hb variants. Unfortunately, neither affinity chromatography nor fructosamine was available in Kaohsiung Veterans General hospital. Hence, a lower target HbA1c range should be re-established in this patient for long-term monitoring glycemic control.

It is essential that clinical laboratories be aware of the limitations of their HbA1c assay methods as well as the importance of visual inspection of ion exchange chromatograms to detect abnormalities (extraordinary peaks or non-separation of each peak) caused by Hb variants. The samples of patients with suspected Hb variants should be analyzed by a method based on a different assay principle, preferably a boronate affinity HPLC. These results also underline the need for additional investigations of interference caused by Hb variants in all newly developed HbA1c assays.

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# 臨床上無症狀的變異血色素(血色素J)患者產生 極低的糖化血色素值——一個病例報告

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## 摘 要

糖化血色素是目前最能準確反映過去二至三個月血糖控制情況的數據。根據美國糖尿病學會的建議，所有糖尿病患者必須定期檢驗糖化血色素，並且控制在7%以下，以預防慢性併發症產生。我們報告一位71歲的第2型糖尿病患者，檢驗出極低之不相符的糖化血色素值，經血色素電泳分析確認變異血色素(血色素J)的存在。藉此病例糖化血色素之分析，強調各臨床實驗室了解其HbA1c檢驗方法的限制，還有靠目視檢查離子交換色彩層析譜來發現因變異血色素而產生不正常的重要性。