Hemoglobin A₁c and Diabetes Mellitus

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ABSTRACT

HbA₁ is the result of the non-enzymatic glycosylation of HbA. HbA₁ reflects the integrated blood glucose level that prevailed over a period of weeks; it thus has clinical application in the assessment of the long-term blood glucose control. This ability to determine easily the long-term blood glucose should enable the clinical investigator to gain insights into the relationship of glycemia and the complications resulting from long-standing diabetes mellitus.

Introduction

Diabetes mellitus is one of the most common diseases, affecting one to five percent of the total population. It is estimated that in the United States alone, over 200,000 new cases are discovered every year.

The treatment of diabetes aims to normalize fat, protein and carbohydrate metabolism. In the overt diabetic, control of excessive fatty acid oxidation and mobilization takes precedence, but limiting hyperglycemia, including urinary caloric loss, and restoring protein losses are also vital considerations.

In contrast, the long term treatment of diabetes has emphasized control of the blood glucose level—not only to prevent the acute complications of ketosis and hyperglycemia but also perhaps in minimizing long-term diabetic complications. It is to this latter aspect, the relation between glucose levels and long-term diabetic complications, that HbA₁ levels may prove to be of considerable use. One of the major problems in determining whether or not the long-term complications of diabetes is glucose-related has been the lack of a measure of long-term glucose control. The findings that HbA₁ is a satisfactory indicator of the integrated blood glucose level over prolonged periods of time has thus aroused considerable interest.

The major form, over 90 percent, of adult hemoglobin is HbA (HbA₁₁) with a subunit structure of α₂β₂. Most of the remainder consists of HbA₁α (1 to 2 percent), HbA₁β (1 to 2 percent) and HbA₁ε (4 to 6 percent). These latter minor hemoglobins can be separated from HbA by various chromatographic procedures. At a slightly acidic pH, the minor hemoglobins are more negatively charged than HbA. Thus, when hemolysate is applied to a cation exchange resin eluted with buffer, pH 6.7, the first fractions will contain HbA₁α.
which is followed by HbA_{1b} and later HbA_{1c}.

HbA_{1c} was first identified by Allen et al.\textsuperscript{1} It was found by Holmquist and Schroeder\textsuperscript{16} to differ from HbA by an unidentified compound bound to the terminal n-\textsuperscript{ogen} of the β-chain. Bookchin and Gall\textsuperscript{3} then showed that the N-terminal compound of the β-chain possessed an added hexose, identified by Bunn et al.\textsuperscript{4} as glucose and mannose in a ratio of 3:1. Glycosylation is not limited to the N-terminal position of the β-chain, since it is also known to link to the ε-amino groups of lysine residues in both α and β chains.\textsuperscript{12}

It has been shown that HbA_{1c} is the result of the glycosylation of HbA—the reaction occurring in the erythrocyte during its stay in the peripheral circulation.\textsuperscript{10} The glycosylation reaction is non-enzymatic, and there is convincing evidence that its rate is dependent on the blood glucose level and the duration of exposure to blood glucose. Thus, the HbA_{1c} content of aged erythrocytes is significantly higher than that from young erythrocytes and this was observed for both healthy subjects and diabetic patients. Similarly, the level of HbA_{1c} is lower in patients whose erythrocytes have a shortened life span owing to hemolysis.\textsuperscript{5}

The increased glycosylation of hemoglobin under conditions of a raised blood glucose is most easily observed in the accumulation of HbA_{1c},—this being the most abundant of the minor hemoglobins. However, an increase in glycosylation of the ε-amino lysine residues of the α and β chains has also been noted.

In addition to the findings of an increase in glycosylated hemoglobin, changes in other glycoproteins have been reported in diabetes. There is evidence that the thickened basement membrane of diabetic glomeruli is composed of abnormal glycoprotein selectively enriched with carbohydrate.\textsuperscript{2} Further, quantitative changes in fibrinogen and von Willebrand factor, also glycoproteins, occur with diabetes.\textsuperscript{7}

### HbA_{1c} and Diabetes Mellitus

The findings of increased amounts of a minor hemoglobin component in diabetes antedated the identification of HbA_{1c}. Huisman and Dozy\textsuperscript{16} in 1962 were the first to report a 2 to 3 fold increase in the minor hemoglobin component in four diabetics. An independent study by Rahbar\textsuperscript{18} also found an elevation in a minor hemoglobin in two diabetics, and he later confirmed this in 47 others. The minor hemoglobin component was subsequently identified as HbA_{1c}.

Amongst the early clinical studies of HbA_{1c} levels in diabetic patients was that by Koenig et al.\textsuperscript{17} They found that the concentration of HbA_{1c} appeared to reflect the mean blood sugar level over the previous weeks. Consistent with these findings have been those of other investigators who found that the highest correlation between HbA_{1c} and urinary glucose was with urinary glucose excreted two months prior to the HbA_{1c} determination.\textsuperscript{5,11} These data suggested that glycosylated hemoglobin levels reflect the integrated glucose concentration over the previous weeks and hence an index of long-term blood glucose control. Thus its potential use, for studies to determine whether or not long-term diabetic complications are a consequence of poor metabolic control, became apparent. There have been no reports as yet of prospective long-term studies. Trivelli et al\textsuperscript{19} did not find a difference when isolated HbA_{1c} levels of diabetic patients with or without long-term complications were compared; studies of long-term complications included peripheral vascular disease, retinopathy, neuropathy and cerebrovascular accidents. Similar results were obtained by Coller et al\textsuperscript{7} in studies on diabetic retinopathy.

Certain known reversible biochemical phenomena and HbA_{1c} in diabetes have been studied. Fasting plasma glucose and the mean value obtained from frequent blood glucose estimations over 24 hour
periods showed excellent correlations with HbA1 levels. Diabetics are prone to anemia, infection and thrombotic episodes with macrovascular and microvascular consequences. These complications may be partly due to the specific abnormalities of leucocyte and platelet function that have been observed in diabetic patients. To determine whether or not these complications were related to the degree of blood glucose control, tests on the formed elements of blood were compared with HbA1c levels. It was found that the erythrocyte half-life, leucocyte adherence and the secondary lag phase time of epinephrine-stimulated platelet aggregation showed a good correlation with HbA1c. Further, correction by strict carbohydrate control over weeks lowered the HbA1c levels and this was accompanied by a reversal of the abnormal hematological tests.

The metabolic derangements observed in newborn infants of diabetic women have been attributed to the mothers' chronic hyperglycemia. Since HbA1c reflects the chronic glycemic state, it has been studied in such pregnancies. The results showed that when corrected for gestational age, the relative birth weights correlated in a significant linear manner with maternal HbA1c levels. The authors concluded that HbA1c levels could be of diagnostic importance for predicting fetal size. Also of interest is the finding that the pregnant diabetic has a lower than expected concentration of HbA1c. This has also been observed in pregnant monkeys (female macaque monkeys). This lowered HbA1c level of pregnancy might be due either to lower mean blood glucose values during pregnancy, a hormonal effect or a decreased erythrocyte survival.

**Glycosylated Hemoglobin and Hemoglobin Function**

Doubling or even tripling of the HbA1c content of erythrocytes, as frequently observed in diabetic patients, may promise oxygen delivery. Ditzel and Standl have shown that the P50 at the in vivo pH of whole blood of non-acidotic diabetic patients is significantly lower than normal. This decrease occurs because the binding of 2,3-diphosphoglycerate (2,3-DPG), an important intracellular red cell glycolytic intermediate, to HbA1c is inhibited. This phenomenon has been explained by steric interference of 2,3-DPG by the N-terminal β-chain glucose of HbA1c being at a position in the hemoglobin molecule where this ligand would normally bind and exert its effect. Ditzel and Standl have suggested that this increase in oxygen affinity is responsible for tissue hypoxia and, if chronic, could lead to long-term microvascular complications. A similar finding of an increase in 2,3-DPG has been observed in macaque monkeys induced with diabetes either by streptozotocin-treatment or pancreatectomy; however, the increase was not considered significant and this may have been due either to the small sample size or a less severe glucose intolerance.

**Methodology**

The current practice in measuring HbA1a, HbA1b and HbA1c is carried out employing cation exchange column chromatography. Since a separate determination for HbA1c probably does not offer any special advantage in the clinical setting, most laboratories measure HbA1c—the sum of the minor hemoglobins. The term HbA1c encompasses the sum of HbA1a + HbA1b + HbA1c. A detailed description of this method has been reported by Gabbay et al and is based on a modification of the original procedure described by Trivelli et al. Cation exchange chromatography is particularly laborious and time-consuming for a service laboratory and more rapid methods have been explored. Davis et al and Cole et al have described the application of high pressure liquid chromatography for measuring HbA1c. More recently, a col-
orimetric method has been employed and in this instance the total glycosylated hemoglobin has been measured; this includes glycosylated HbA in addition to that of HbA\textsubscript{i}.$^{12}$

**Indications and Limitations for the Use of HbA\textsubscript{i}**

The measurement of HbA\textsubscript{i} in the assessment of the diabetic patient has considerable advantages over present tests. In contrast to 24-hour urinary glucose or isolated blood glucose determinations, HbA\textsubscript{i} is not dependent on the accuracy of volume collection and it is independent of the time of day, meals or exercise. It is convenient to be able to describe metabolic control with a single value, so that HbA\textsubscript{i} provides the physician with a definitive end-point towards which therapy needs to be directed. HbA\textsubscript{i} may also be usefully employed as a screening test for those diabetics who appear to be satisfactory but whose metabolic control is actually less than optimal.$^{14}$

However, HbA\textsubscript{i} reflects chronic glycemia so that it cannot supersede the necessity for blood glucose estimations in acute situations. Also, HbA\textsubscript{i} levels do not reflect the occurrence of hypoglycemic episodes. Since the glycosylated hemoglobin in situations of a shortened erythrocyte half-life and/or certain hemoglobinopathies underestimates the degree of chronic hyperglycemia, the application of HbA\textsubscript{i} is limited in patients with hemolysis, $\beta$-thalassemia or HbF.

**References**


