# Standardization of hemoglobinA1c

Two recent articles in IJDDC[1,2] have eluded to a few newer aspects of HbA1c. Since the description of this test[3] and our early work,[4,5] modifying and popularizing the test in India, a few interesting aspects of the test have emerged, necessitating a change in our strategy.

## HbA1c and fluctuations in blood glucose

It is postulated that HbA1c reflects an average blood glucose level, whereby very high and very low values will tend to cancel each other and give a good average.[6,7] Although HbA1c reflects the glycemic status quite faithfully,[8] some exceptions are possible. There are no methods for circumventing this objection completely except the use of continuous blood glucose monitoring for 3-6 days of the month or frequent self monitoring of blood glucose to detect wide fluctuations. It is an important issue as the wide fluctuations have the potential of aggravating the vascular complications.

## HbA1c reflects control of diabetes over the past 2-3 months

It was initially postulated that the HbA1c reflects the status of metabolic control over the past three months. With added data, it became obvious that it reflects the glucose status of the last 1, 2 and 3 - 4 months upto 50%, 40%, 10%, respectively.[9,10] Thus, there is a likelihood of a significant change in HbA1c in 4-6 week of improvement or deterioration of metabolic control. Rapid dissipation of glycated hemoglobin has been described upon instituting rapid metabolic control.[11] Similarly, stress hypoglycemia, although transient, is known to elevate glycated hemoglobin level.[12]

## HbA1c preferentially reflects fasting blood glucose or post prandial blood glucose

HbA1c obviously reflects the overall glycemic status. However, at very high levels (>9%) of HbA1c, there is a possibility of severe global hyperglycemia, that is a high fasting and post prandial blood glucose. However, at low values (<8%), the major contributing factor is the prandial blood glucose.[13,14]

## Effect of erythrokinetics on HbA1c

Anemia of any type during its dynamic phase of either development of anemia or its resolution will alter the erythrokinetics and thereby influence the HbA1c results.

A study in a group of patients with hemolytic anemia suggested that estimation of HbA1c can be utilized to study the progress of anemia and judge the severity of the degree of hemolysis.[11]

## Effect of hemoglobinopathies on HbA1c

Hemoglobinopathies influence the testing of HbA1c by altered electrophoretic or immunological or ionic properties of the abnormal hemoglobin and additionally, by altered erythrokinetics. Thus, the results are vitiated depending upon the method of estimation employed.[15] HbF is the most common hemoglobinopathy in India. As HbF co-elutes with HbA1c, it falsely raises the value of HbA1c in the electrophoretic and ion exchange methodologies. It is recommended that population-specific prevalent hemoglobinopathies should be accounted for while establishing the normative HbA1c data in a community.

## Standardization of HbA1c

International Federation of Clinical Chemistry (IFCC) estimated pure and single molecular species of HbA1c, the values of which are much lower than those reported by the use of regular HbA1c methods earlier certified by the National Glycohemoglobin Standardization Program (NGSP) and extensively used in important clinical studies like DCCT and UKPDS.[16] The IFCC method estimates glycation only at the terminal valine of β-chain. The IFCC reference method involves cleavage of the stable adduct of glucose to the N –terminal valine of the B –chain of hemoglobin by the endoproteinase- Glu-C. The glycated and non- glycated N-terminal hexapeptide so generated are separated by HPLC, followed by quantification by electroionization mass spectrometry or capillary electrophoresis.[17] The NGSP certified a traceable method to estimate glycation of hemoglobin at a few other sites besides the glycation at the terminal valine site. The

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normal values reported by NGSP Certified methods is 4-6% while IFCC method is 2.8-3.8%

The relationship of HbA1c and mean blood glucose has been accepted to be linear, based on a massive body of data in the past, most importantly the seven point capillary blood glucose profile obtained during the DCCT study.[18] However, this relationship is still being studied in depth and likely to be reported soon.

Based upon the data of Rohlfing, it is possible to convert IFCC values to mean blood glucose (MBG)

MBG (mmol/l) = 1.84*IFCC-HbA1c.

The IFCC and NGSP methods have been compared;[19] the following equation permits interconversion: NGSP-HbA1c = 0.915 (IFCC- HbA1c) + 2.15%.

An expert committee, consisting of leading investigators from ADA, EASD and IDF, recommends that all HbA1c instruments should be calibrated to report the results by the IFCC reference range. However, the results should be converted to NGSP method before reporting to the clinicians and patients. Reporting of the results by IFCC method may produce a sense of complacency in these groups, as the values are lower;[20] alternatively, both the values from both the methods should be converted to MBG, as most patients understand the blood glucose values very well. Once the new data regarding the relationship of either NGSP or IFCC - HbA1c methods are available, this conversion will have great validity. Most acceptable method of reporting to the clinicians would be in terms of NGSP traceable HbA1c and MBG.

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Editor, IJDDC.

**DOI:** 10.4103/0973-3930.66500