Glycohaemoglobin - a crucial measurement in modern diabetes management.
Progress towards standardisation and improved precision of measurement

Consensus statement from the Australian Diabetes Society, Royal College of Pathologists of Australasia and Australasian Association of Clinical Biochemists

Peter G. Colman FRACP, MD
Director, Department of Diabetes and Endocrinology, Royal Melbourne Hospital

G. Ian Goodall BSc, FAACB
Unit manager - Special Chemistry, Austin and Repatriation Medical Centre

Peter Garcia-Webb MD, FRCPA
Chemical Pathologist, St. John of God Pathology, WA

Paul F. Williams MSc, PhD
Principal Hospital Scientist, Royal Prince Alfred Hospital

Marjorie E. Dunlop MSc, PhD
Principal Research Fellow, University of Melbourne Department of Medicine

Address for correspondance:
Dr. Peter Colman
Department of Diabetes and Endocrinology
Royal Melbourne Hospital
PO Box 3050,
Victoria, Australia.
Ph: 03-9342-7428
FAX: 03-9342-7898
email: peterc@nursing.medrmh.unimelb.edu.au

Summary

The Diabetes Control and Complications Trial has focussed increased attention on the importance of glycaemic control in preventing or retarding the progression of complications in patients with diabetes. Regular measurement of glycohaemoglobin is now recognised as an essential adjunct to self blood glucose measurement assisting in the achievement of the best possible glycaemic control. However, clinicians using glycohaemoglobin assays should be aware of several potential problems which can confound the interpretation of the glycohaemoglobin result.

Firstly, there are currently four different assay principles (ion-exchange chromatography, electrophoresis, affinity chromatography and immunoassay) and approximately 20 different methods used to measure glycohaemoglobin which measure different glycated products, report different units and which can produce numerically different results for the same specimen. Standardisation, which will lead to all assays reporting results in a standard unit, %HbA1c, is recommended and should be in place within the next one to three years. In the interim, clinicians using glycohaemoglobin assays should be aware that the ranges indicating good and poor glycaemic control can vary markedly with the different glycohaemoglobin assays.

Secondly, the reproducibility of some of the assays used may be substandard, rendering them unable to provide definitive evidence of changes in glycaemic control and hence confounding the utility and interpretation of results. Indeed some assays may be so imprecise that they are unable to reliably separate patients with good and poor control. Clinicians should be aware that assays with high precision are the most desirable and ideally assays with an interassay coefficient of variation of less than 3% are likely to be more clinically useful.

What is glycohaemoglobin and why should we measure it?

Glycohaemoglobin is formed by a non-enzymatic interaction between glucose and the amino groups of the valine and lysine residues in haemoglobin. Formation of glycohaemoglobin is irreversible and the level in the red blood cell depends on the blood glucose concentration. Thus, measurement of glycohaemoglobin, which was first introduced in the 1970's, provides a measurement of glycemic control over time, which has been proven to evoke changes in diabetes treatment resulting in improved metabolic control (1). It is now accepted as a unique and important index in diabetes management reflecting the degree of metabolic control and was a major determinant of the landmark Diabetes Control and Complications Trial (DCCT).

In the DCCT trial (2) 1441 patients with insulin-dependent diabetes were randomised to either intensive treatment and monitoring (usually with 4 insulin injections/day or pump treatment), with the aim of achieving normoglycaemia, or to conventional treatment (usually with one or two injections/day). The effectiveness of intensive therapy was reflected in clear differences in mean blood glucose levels and glycohaemoglobin between the 2 groups. For instance, the intensive treatment group achieved a mean daily blood glucose of 8.6 mmol/L and a median HbA_{1c} of 7.2%, compared with the conventional group which achieved a mean blood glucose of 12.8 mmol/L and a median HbA_{1c} of 8.9%. These differences in control were maintained over a mean period of 6.5 years and were associated with a 35-76% reduction in the development and progression of retinopathy, nephropathy and neuropathy. The findings of this study have focussed increased attention on the critical importance of metabolic control in patients with diabetes (3)

Based on the findings of the DCCT, it is now possible for doctors caring for patients with diabetes to establish targets for glycemic control, which are based on observed outcomes, and which, if met should minimise the development of complications. Inevitably, because glycohaemoglobin measurements reflect an integrated view of glycaemic control over time, the patients and their carers will place increasing reliance on the glycohaemoglobin result. It

is thus very timely to evaluate the types of assays available, the moves toward standardisation of the reporting units and the precision and reproducibility of current assays.

At the outset it must be realised that in the DCCT all the glycohaemoglobin measurements were performed using the same closely standardised method. Unfortunately, in Australia there are currently 4 different assay principles and approximately 20 different methods used for glycohaemoglobin most of which are not standardized between laboratories.

Types of assays available:

The four different methods used to measure glycohaemoglobin are - ion-exchange chromatography, electrophoresis, affinity chromatography and immunoassay. The methods measure slightly different glycated products and use at least three different units for reporting the results [% HbA1c, %HbA1 and % total glycohaemoglobin (GHb)]. In addition they can produce different numerical values for the same patient specimen.

This was demonstrated in a recent study in which four whole blood samples with HbA1c levels of 5.1% (representing non diabetes), 6.7% (representing excellent glycaemic control), 8.5% (representing moderate glycaemic control) and 11.4% (representing poor glycaemic control) were distributed to 29 laboratories in Victoria for glycohaemoglobin determinations (4). The range of values obtained for the non-diabetic (4.1-6.8%), good control (5.1-9.3%), moderate control (6.7-11.9%) and poor control (10.1-17.3%) specimens demonstrated extensive overlap between samples which were designed to represent patients with markedly different degrees of glycemic control (figure 1). At present it appears impossible to compare the results from two different laboratories; this can be confusing not only for patients but also for their carers. The use of laboratory-specific reference ranges makes possible a means by which results from different laboratories can be compared. However, the data used to derive such ranges are arbitrary and the categories into which different glycohaemoglobin levels are divided may be misleading.

Importance of reproducibility of measurement

A major use of the glycohaemoglobin assay is to assess changes in metabolic control that follow an alteration in treatment. The ability of any assay to reliably detect a change in the analyte measured depends on its reproducibility (or the ability of the assay and laboratory to get the same answer for the same sample each time). Reproducibility is normally expressed as the coefficient of variation (CV) of an assay. The CV is obtained by measuring the same sample at least 20 times in different assay runs and calculating the mean and standard deviation (SD) of the measurements; the CV is calculated by dividing the SD by the mean and expressing the result as a percentage. An assay with a high CV suffers from poor reproducibility and is unable to determine if glycohaemoglobin levels have changed in different samples. Laboratories normally make decisions to accept an assay for reporting results if the result for quality control samples fall within a range of mean $\pm$ 3SD. The imprecision of measurement of patient samples will be similar to that of the quality control samples. For example, an assay with good precision (3%CV) at a patient level of 7% HbA1c would have 3SD ranges of 6.37 - 7.63 %HbA1c; at a patient level of 9% HbA1c the 3SD range would 8.19 - 9.81 %HbA1c. These two values can clearly be separated. In contrast an assay with poor precision (6% CV) at a patient level of 7% HbA1c and 9% HbA1c would have 3 SD ranges of 5.74 - 8.26 %HbA1c (for the 7% level) and 7.38 - 10.62 %HbA1c (for the 9% level). Obviously, these levels cannot be differentiated by an assay with this level of precision.

How well are assays in Australia performing?

The Royal College of Pathologists of Australasia/Australasian Association of Clinical Biochemists Chemical Pathology Quality Assurance Programme provides external quality control samples for laboratories in Australia reporting glycohaemoglobin levels (5). The program runs on a 6 monthly cycle with 2 random samples analysed per month from duplicates of six levels of lyophilised whole blood samples. The use of lyophilised samples raises the possiblity of matrix effects, which could lead to some variation. However, a recent study has excluded this as a complicating factor (6).

At the intensive control outcome level for the DCCT of 7.2% HbA1c, Australian laboratories reported HbA1c assay values between 6.0 and 9.0% HbA1c, while the range of values reported for all units (%HbA1c, %HbA1 and % total GHb) was between 6.0 and 12.6%. At the conventional control level for the DCCT trial of 8.9% HbA1c, laboratories reporting HbA1c units reported values between 7.4 and 11.0%, while the range of values for all glycohaemoglobin units was 7.4% to 16.4%. For Australian laboratories the methodological interlaboratory CV obtained varied between 1.6 and 8.9% for the most common assays. The overlap between values obtained for these samples epitomizes the problems currently facing clinicians in interpreting glycohaemoglobin levels and changes in levels reported by different laboratories.

In order to critically evaluate changes in HbA1c it is important that the precision of individual laboratory assays for glycohaemoglobin be known. For example the difference between the intensive and the conventional treatment groups in the DCCT was only 1.7% HbA1c, thus it is self evident that any assay used should at least be able to reliably detect a difference of this order. In order to achieve this assays must have a CV of <3.0%. Table 1 shows the effects on imprecision of varying CVs at the mid point (8.05 %HbA1c) of the two DCCT groups (intensive group 7.2% HbA1c, conventional group 8.9% HbA1c). With most laboratories using the $\pm$ 3SD limits to accept or reject assay runs, clearly glycohaemoglobin assays with CVs close to 3% are necessary to differentiate the two DCCT group means for a patient who lies at the mid point. At 3% CV the mean $\pm$ 3SD range of values for a patient with a true HbA1c level of 8.05 %HbA1c would be 7.33 to 8.77 %HbA1c. Therefore, even glycohaemoglobin assays with 3% CV are not ideal. Realistically, however, only HPLC assays can currently achieve such precision.

We recommend that the CV of the assay currently being used by the reporting laboratory be made available to carers using glycohaemoglobin measurements. This will allow them to determine if the assay has the ability to differentiate between reported levels. Reference

laboratories in the International Federation of Clinical Chemistry (IFCC)/American Association of Clinical Chemistry (AACC) International Standardization Programme (see below) must be able to achieve a CV of less than 3% at HbA1c levels of 6% HbA1c and 9% HbA1c. Manufacturers assays should be able to achieve a CV of less than 5%. Currently poor GHb assays are unable to achieve these limits.

Progress towards standardization

Clearly standardization is critical to allow comparison between results obtained in different laboratories using different methods. A working party of the IFCC and AACC is coordinating an international effort by which all methods will be standardized to a designated method. This will be performed at the manufacturer level. Glycohaemoglobin analyser and kit manufacturers will have their assays standardised by Reference Laboratories established and monitored monthly by the IFCC/AFCC working party. Thus ultimately all laboratory methods will report their results in %HbA1c units which have been standardized against the DCCT method (7). Patients and carers will then be able to directly compare their level of glycemic control against the enormous amount of data obtained by the DCCT trial on the onset and incidence of diabetes related complications.

Interim Recommendations

(1) The terminology to be used for the assay is glycohaemoglobin (GHb) assay*

(2) The unit of measurement for GHb assays should be reported as %HbA1c (DCCT equivalent).

(3) Other units such as % total GHb or %HbA1 should not be used. Assays producing these units should be converted to %HbA1c reporting units.

(4) Assays with low imprecision are highly desirable. The IFCC/AACC are currently recommending between run coefficients of variation of less than 5% for manufacturers of kits and instruments. However, between run coefficients of variation of less than 3% are far more clinically useful and therefore desirable.

*Recommendation from the combined meetings of the IFCC Working Group on HbA1c standardisation and the AACC Subcommittee on Glycohaemoglobin.

References

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TABLE 1: Effect of imprecision on a patient with HbA1c value of 8.05 (the mid point of the range between the intensive and conventional control ranges in the DCCT).

CV%	SD	3SD	3SD RANGE
2.0	0.16	0.48	7.57 - 8.53
3.0	0.24	0.72	7.33 - 8.77
3.7	0.30	0.89	7.16 - 8.94
4.0	0.32	0.97	7.08 - 9.02
5.0	0.40	1.21	6.84 - 9.26
7.5	0.60	1.81	6.25 - 9.86
10.0	0.81	2.42	5.65 - 10.47

Figure Legend

Horizontal axis shows the four samples from patients with differing degrees of diabetes control. The closed circles represent individual laboratory results for each sample and the open circles the notional target value. The notional target value was set by the Biorad Diamat in a laboratory where the assay was referenced against the DCCT method. The variation in values for the major types of methodology is shown in the six columns for each of the four samples. Columns are numbered from left to right.

1: HPLC (cation exchange, measuring HbA1c)

2: Immunoassay (measuring HbA1c)

3: Ion Exchange Chromatography (Manual assay, measuring HbA1c or HbA1)

4: Affinity Chromatography (measuring total GHb but expressed as either total GHb or %HbA1c)

5: Electrophoresis (measuring HbA1c or HbA1)

6: Low Pressure Liquid Chromatography (measuring HbA1c and including HbF).