

## 15. MONITORING OF DIABETES MELLITUS

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Diabetes mellitus has become a major health problem worldwide, reaching epidemic proportions in many developing countries, as well as in minority groups in developed countries. Worldwide projections suggest more than 220 million people with diabetes by the year 2010 and the majority of these (approximately 210 million) will have type 2 diabetes. Health care costs due to diabetes are approximately 14% of the total health care budget, with half in direct costs, and they too are projected to rise considerably.

Type 1 diabetes is accompanied by long-term microvascular and macrovascular complications, the primary causes of morbidity and mortality in these patients. Diabetic nephropathy, as the single most common cause of end-stage renal disease, accounts for over one-third of all cases. Type 2 diabetes mellitus is associated with increased cardiovascular and overall mortality. In fact, type 2 diabetic patients diagnosed before 70 years have only 70% of the life expectancy of non-diabetics. Epidemiological data suggest that classic cardiovascular risk factors like hypercholesterolemia, hypertension, and smoking do not account for the excess risk of cardiovascular morbidity and mortality in type 2 diabetes mellitus.

Regular monitoring of the glycaemic status of the diabetic patient is considered a cornerstone of diabetes care. Results of monitoring are used to assess the efficacy of therapy and to guide adjustments in lifestyle to achieve best possible glucose control.

The tests used most widely in monitoring the glycaemic status of people with diabetes are blood glucose and glycated haemoglobin.

### 1.1. A: Glucose testing

Within the last years, self-monitoring of blood glucose (SMBG) has revolutionised management of diabetes. Using SMBG, patients with diabetes can work to achieve and maintain specific glycaemic goals. There is now broad consensus on the health benefit of near-normal blood glucose levels and of the importance, especially in insulin-treated patients, of SMBG in treatment efforts designed to achieve such glycaemic goals.

#### 1.1.1. Self-monitoring of blood glucose

It is recommended that most individuals with diabetes should attempt to achieve and maintain blood glucose levels as close as normal as possible. Treatment programs should encourage SMBG for routine daily monitoring. Frequency and timing of glucose monitoring depends on the needs and goals of the individual patient and varies from 7 times daily in type 1 diabetics on multiple insulin injections to 4 times weekly in type 2 diabetics on diet.

Because the accuracy of SMBG is instrument- and user-dependent, it is important to evaluate the patient's monitoring technique, both initially and at regular intervals. In addition, because laboratory methods mostly use methods measuring venous plasma glucose, many

blood glucose meters approved for home use now calibrate blood glucose readings to plasma values. But, in general, plasma values are 15% higher than whole blood glucose values. It is therefore crucial that the patient know whether their glucose monitor provides whole blood or plasma values.

### **1.1.2. Laboratory measurement of glucose**

Of course, blood glucose testing (e.g., finger-stick whole blood or venous plasma) should be available to all health care providers and patients. However, with the availability of SMBG, routine laboratory-based blood glucose testing is no longer the predominant way of assessing glycaemic control. Regular comparisons between results from the SMBG and the laboratory are useful to assess the accuracy of patient's results. If such testing is performed by health-care providers using portable capillary blood testing devices rather than standard laboratory methods, then rigorous quality control is needed.

## **1.2. B: Measurement of glycohaemoglobin**

Of the several pathogenic mechanisms by which hyperglycaemia may lead to altered tissue structure and function, non-enzymatic glycosylation (encompassing the attachment of free aldehyde groups of glucose or other sugars to the unprotonated free amino groups of proteins) causes altered structure and function of several soluble and insoluble proteins. Lens crystalline and serum albumin have altered conformations and glycated haemoglobin (HbA1c) shows significantly altered functional properties. Non-enzymatic glycosylation changes also the structure and function of isolated basement membrane components. The accumulation of glycation products and the accompanying structural modifications correlate with the development of functional complications of diabetes. These changes in tissue structure and function are slow and cumulative, resulting in a long time lag between the diagnosis of diabetes and the onset and progression of the complications of diabetes mellitus.

Haemoglobin is one of many proteins that undergo non-enzymatic glycosylation and glycated haemoglobin (GHb) is a general term for haemoglobin non-enzymatically glycosylated by glucose. Rahbar first described GHbs in 1968 as diabetic haemoglobins. Potential glycation sites of the haemoglobin A molecule include the N-terminal amino acid valine of the four polypeptide chains and all the free  $\epsilon$ -amino groups of lysine residues. The predominant glycation site is the N-terminal valine residue of the b-chain, accounting for approximately 60% of bound glucose. Thus, HbA1c is defined by the International Federation of Clinical Chemistry (IFCC) as HbA0 irreversibly glycosylated at the N-terminal valine of the b-chain. Other glucose molecules can be bound to one or more of the 44 glycation sites at the  $\epsilon$ -amino groups within the haemoglobin molecule or at the N-terminal valine of the a-chain.

HbA1, more negatively charged than HbA0, may be detected by cation-exchange chromatography and includes HbA1a, HbA1b and HbA1c, which are named in order of their elution from the column. Of these, HbA1c represents the most prevalent glycated species. Total GHb refers to all GHb species that are measured by affinity chromatographic methods. Since erythrocytes are freely permeable to glucose, the rate of formation of glycated haemoglobin is directly proportional to the ambient glucose concentration in which the erythrocyte circulates and to the duration of exposure. In addition, as the post-synthetic modifi-

cations of HbA to form GHb are essentially irreversible, the level of GHb is a reliable integrated measure of the average blood glucose concentration during the preceding 120 days.

Clinical GHb testing became widely available in the early 1980s, and thus objective measurement of long-term glycaemic status became possible. Measurement of GHQ is recommended by organisations like the American Diabetes Association and is widely used in clinical practice to monitor glycaemia in diabetic patients. In addition, it serves as a key predictor of the risk to develop diabetic complications. Most important, the measurement of GHb served as the primary parameter of glycaemic control in major clinical trials (especially the DCCT and UKPDS) which addressed the efficacy of intensive diabetic therapy in preventing or delaying long-term diabetic complications. In addition, knowledge of GHb levels appears to alter the behaviour of health care providers and/or patients, in turn improving glycaemia and lowering GHb values.

### **1.3. Glycosylated haemoglobin and diabetic complications**

Only in the last decade have the DCCT and the UKPDS clearly demonstrated that improved glycaemic control reduced the development and progression of several microvascular and macrovascular complications in both type 1 and type 2 diabetes mellitus. Of interest both studies utilised the same ion exchange HPLC method. The key findings from these and other selected studies are summarised below:

#### **1.3.1. A: Retinopathy**

In the DCCT, with 1441 type 1 diabetic participants, intensive therapy with a mean HbA1c of 7.2% reduced the risk of development of retinopathy in the primary prevention cohort and lowered the risk of progression of retinopathy in the secondary intervention cohort by 76% and 57%, respectively, compared to the conventionally treated group with a mean HbA1c of 9.1%. In the UYPDS, 3867 newly diagnosed type 2 diabetics were followed over 10 years. Compared with the conventional group with a mean HbA1c of 7.9%, the intensively treated group had a 25% risk reduction in microvascular complications, including the need for retinal photocoagulation. In WESTDR, with 1210 younger onset and 1780 older onset diabetic patients, HbA1c at baseline was a significant predictor of incidence and progressions of proliferate retinopathy after adjusting for duration of diabetes. HbA1c levels (divided into both quartiles and deciles) correlated with a consistent increase in retinopathy from the lowest to highest quartile with no evidence of a threshold effect. Furthermore, the WESTDR investigators estimated that a 1.5% point decrease in HbA1c would lead to a 24-33% decrease in the 10year incidence of proliferate retinopathy.

#### **1.3.2. B: Nephropathy**

In the DCCT, with the two cohorts combined, intensive therapy reduced the incidence of microalbuminuria (urinary albumin excretion of >30mg/24hrs) by 39% and clinical grade albuminuria (urinary albumin excretion of > 300mg/24hrs) by 54%. In the Wisconsin cohort, 28% of all younger and 36% of all older patients developed gross proteinuria, and 7% of all younger and 2% of all older patients developed renal failure. In this study, compared to patients in the lowest quartile, patients in the highest quartile of HbA1c had a 2- to 4-fold in-

creased risk of both proteinuria and renal failure.

### **1.3.3. C: Macrovascular disease**

In the UKPDS, the intensively treated group had a 10% lower risk for any diabetes-related death and 6% lower risk for all-cause mortality, when compared with the conventionally treated group. In the Wisconsin cohort studied by Klein, the hazard ratio for dying was 1.9-fold greater for patients in the 4th quartile of HbA1c levels, relative to the 1st quartile. Similarly, Ravid et al. found high glycated haemoglobin to be associated with a 15-fold greater risk of cardiovascular morbidity and mortality, compared to low. HbA1c levels at baseline were a strong predictor of cardiovascular risk factors, cardiovascular events, stroke, and overall mortality in other studies.

### **1.4. The measurement of glycosylated haemoglobin**

Despite the overwhelming evidence that GHb measurements should be used to guide the therapy of diabetes, the test appears to be under-utilised clinically. One major reason subsumes the many analytical methods (more than 20), most of which measure different combinations of chemically modified haemoglobins. The National Glycohemoglobin Standardization Program (NGSP) and the IFCC working group have continuously improved the standardisation of glycohaemoglobin measurements. While the method used in the DCCT study has been proposed as the comparison method, against which most assays should be standardised, there is no universally accepted reference method. For example, the HbA1c result by HPLC ion exchange is only about 60% specific! Despite the challenges with glycosylated haemoglobin standardisation, the clinical studies, irrespective of the different analytical methods employed and the variation in the values for a “normoglycaemic” reference population, clearly showed that poorer metabolic control was associated with an increased risk of microvascular and macrovascular complications of diabetes mellitus.

### **1.5. New developments in estimation of glycosylated haemoglobin**

Recently a method utilising electrospray ionisation-mass spectrometry has been developed as candidate reference methods for estimation of HbA1c, following a trend for many specific reference methods in clinical chemistry to be based on HPLC-MS. The method by Kobold et al. analysed endoproteinase Glu-C digests of whole blood samples. Endoproteinase Glu-C cleaves N-terminal segments of the b chains between the two glutamic acid residues at positions 6 and 7, with the resulting fragments containing only a single glycation site at the chain N-terminal valine. By this approach, interference by carbamylated and acetylated N-terminal species and by the dimer of glycated a-chain and nonglycated b-chain is excluded. Overall, the measure of glycation at the N-terminal valine of the b-chain is more specific, and a proposed reference system demands an exact knowledge of the analyte to be measured.

Since the development of complications is linked to the accumulation of glycation adducts in tissue proteins; any analytical method that serves as an index of measurement of levels of glycation should clearly be used to guide therapy in diabetes. Although the ion-exchange method does not meet contemporary standards for accuracy, immensely valuable prognos-

tic information has been gathered with this procedure over nearly two decades of observing the 1400 to 3800 subjects, respectively, in the DCCT and the UKPDS. Other important studies, both prospective and retrospective, have used either the ion-exchange method or have been standardised against the DCCT method for estimation of GHb. Since it is clear that near-normal glycaemic control is necessary to prevent development and progression of complications, and since it is difficult to reverse complications, one cannot justify a clinical trial with another method to confirm the efficacy of glycaemic control upon diabetic complications, as demonstrated by the DCCT and UKPDS. Moreover, from the results of these clinical trials it would be unethical to initiate a new prospective trial with treatment groups having different levels of glycaemic control to test the efficacy of the new reference method for glycated haemoglobin. Therefore, whichever reference method may be adopted, the HbA1c values measured in the DCCT and UKPDS will have to be translated into values based on the new reference systems. Furthermore, this translation must be computationally and efficiently effected, particularly since in some countries federal regulations mandate adequate monitoring of glycaemic control by GHb estimation as a necessary component of management for the patients and their health care providers.

One possible approach to reconcile the values among the methods might include follow-up of a subset of the DCCT and UKPDS subjects with both the ion-exchange method and the new reference method(s), followed by a consensus statement concerning new values to set the standards of glycaemic control. In any case, the transition to new standards must be completed with caution, i.e. only when the new method's efficacy can be compared with the ion exchange method - a procedure used in two major trials and whose values reflect the contemporary clinical standard.

#### **Recommended literature:**

1. The Diabetes Control and Complications Trial Research Group: The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 1993; 329:977-86.
2. UK Prospective Diabetes Study group: Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet* 1998;352: 837-53.
3. American Diabetes Association: Clinical Practice Recommendations 2001. *Diabetes Care* 2001;24 (suppl.1):S1-S133.
4. Krishnamurti U, Steffens MW. Glycohemoglobin: a primary predictor of the development or reversal of complications of diabetes mellitus. *Clin Chem* 2001;47:1157-65.