REVIEW

Current Glycemic Testing: A Review Of Blood Glucose, Hemoglobin A1c, and Glycated Albumin Values

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Glycemic control of diabetes mellitus is currently monitored by a combination of self-monitored blood glucose (SMBG) and hemoglobin A1c (A1c) test results. This review article will briefly cover a number of issues raised in recent reports in conjunction with these methods of diabetes monitoring. Measurement of the percentage of glycated albumin has been proposed as an improved diabetes control index based on levels of short-to-intermediate glycation. We have collected current and historical information on the range of reported values for glycated albumin in normal and diabetic subjects derived from a number of different test methodologies.

Issues in SMBG and A1c Testing

Glycation levels and glycemic control are currently monitored by combining information from two sources of data. SMBG readings generate virtually instantaneous individual data points for blood glucose levels, which are then used to extrapolate glycation from overall trends in blood sugar levels. Although they may not be as accurate as laboratory methods, many current glucose meters meet the International Organization for Standardization guidelines (Saudek et al., 2006). Thus these values, along with the diagnostic ranges leading to complications, are well-established and reliable measurements of the diabetic condition (Guglucci, 2000).

Ideally, this data is then correlated with information on long-term glycation derived from A1c testing, performed every 3-6 months. A number of recent reports question the efficacy and relevance of frequent SMBG monitoring for non-insulin dependent type 2 diabetics (Brownlee and Hirsch, 2006). In these patients (80-90% of diabetic population), the role and frequency of SMBG is not clear, especially in those receiving oral agents or nutritional therapy. Effective action by certain type-2 patients may be confounded by the lack of an insulin-injection cue, thus illustrating the need for a management index reflecting the magnitude of glucose fluctuation over days and weeks. Saudek et al. (2006) recently reviewed glycemic assessment in diabetes and concluded in favor of regular SMBG monitoring, especially for individuals taking insulin. For Type-1 patients they point toward an ultimate goal of replacing SMBG with continuous glucose monitoring. For type 2 patients they admit less definitive evidence for SMBG improving glycemia, citing education level and action response as important influences on efficacy, even in randomized controlled trials.

Correlation of the results of SMBG testing with A1c is facilitated by the linear correspondence between mean plasma glucose and A1c, for which tables are widely published. However, the measurement of fasting plasma glucose (FPG) tends to underestimate the A1c value and by itself may not be a reliable predictor of long-term glycemia. The American Diabetes Association currently recommends a target A1c of less than 7%. Since it is now known that a change of about 25-35 mg/dl in the average blood glucose concentration corresponds to a change of about 1% in A1c, there is an increasing need for precision in the A1c measurement. For example, the Food and Drug Administration has approved an antidiabetic agent on the basis of a 0.8% lowering of glycohemoglobin (Cohen and Clements, 1999).

Confidence in A1c and its central role in diabetes management has been discussed by Jeffcoate (2004) who pointed out three areas of uncertainty. In order of importance they are (1) clinical variability, (2) biological variability, (3) analytical variability. The latter has recently been addressed in part by reference method standardization. However, there remains the discrepancy from two different reference value approaches resulting in different A1c values. The former two need a great deal of work, especially within the context of diabetic self-care. Of particular interest are recent reports by Moriyama et al. (2005), and Monier et al. (2006) where A1c failed to track the diabetic episode, but results based on a shorter-term glycation index did.

The Intermediate Glycation Index

In spite of its checkered success, the fructosamine (FA) assay made those who care for diabetics aware of the benefits of a reliable, inexpensive, intermediate term glycation index to improve diabetes management and potentially to screen for the disease among millions of prediabetics. A plethora of reports have indicated that the glycated albumin (GA) test, hitherto relegated to specialty laboratories, has the potential

to be a more reliable intermediate tracker of diabetic management than FA (Winocour et al., 1989; Schleicher et al., 1993; Hicks et al., 2001). In addition to the greater specificity of the test, which avoids the problems observed in the FA assay, the result, when rendered as a percentage of total albumin, provides baseline information not available through a measurement of FA concentration.

Forty-eight large clinical laboratories from major urban areas across the U.S. with the potential for performing a glycated albumin test were recently contacted. Only two stated that they perform the test themselves--ARUP (Utah) and Quest (California)--and another 13 stated that they offer the test and send samples to either ARUP or Quest. The remaining 33 labs stated that they did not offer the GA test. The normal GA reference ranges stated by these two labs are 0.6-3.0% (ARUP) and 0.8-1.4% (Quest). In contrast, recent reports from Japan that discuss GA testing (Kouzuma et al., 2002, 2004; Moriyama et al., 2005) cite normal and diabetic GA percentages that are an order of magnitude higher than those cited by the two American specialty labs.

In an effort to review and evaluate the methodologies for testing glycated albumin and the values generated, we have considered twenty-six published reports regarding normal and diabetic GA values, as well as the information regarding current clinical laboratory practice. Table 1 presents pertinent information from the literature relevant to GA measurement, associated technology, patient information, authorship, and date, with the entries arranged in ascending order of GA%. The purpose of this exercise is twofold: (1) to understand the range of reported values encountered clinically in normal and diabetic populations and (2) to identify those factors strongly associated with generated GA values. Nearly half (12/26) the reports describe normal GA values of 2.6% or lower, several (7/26) reports indicate normal values in the range of 5-9%, and others (7/26) report normal values in the range of 10-20%. Overall, typical diabetic GA values are 2-5 times normal values in a given report. Clearly reference method standardization is lacking for the GA test.

Quest Labs simply stated that they used affinity clhromatography for determining glycated albumin, while ARUP specified boronate affinity chromatography, and further indicated that quantification of albumin was by a turbidimetric immunoassay. In Table 1 affinity chromatography separation appeared to be associated with great disparity in both normal and diabetic GA%. Reported normal values ranged from means of 0.6% to 8.6%, and corresponding mean diabetic values ranged from 1.4% to 16.59%. Throughout the table, older reports (1980-1985) are associated with higher GA values than more recent reports, which suggest that lower values can represent a refinement in technique. We considered that an explanation of the disparate values might be found in the various techniques to quantify albumin once separation was performed. However, no pattern could be discerned to explain the disparity by a single technique. Using the colorimetric quantifier bromcresol green (BCG) as an example, gel electrophoresis + BCG yields low GA reference values; use of affinity columns + BCG yields higher values; and use of an enzymatic method + BCG yields the highest values.

Rendell et al. (1985) reported that aminophenylboronic acid affinity chromatography reliably distinguished diabetics from nondiabetics in contrast to thiobarbituric acid (TBA) colorimetry. However, Johnson and Baker (1988) showed that TBA and boronate chromatography underestimate GA values, using radiolabeled glycated human albumin as a test standard. Both reports associate TBA with the susceptibility of colorimetric methods to biochemical interference agents. However, the results of boronate-affinity chromatography may be skewed because of an imprecise association of glycated molecules vis a vis binding sites on the resin. This may explain why the boronate-based methodology of ARUP yields a broader normal GA range (0.6-3.0%) than the methodology used by Quest (0.8-1.4%). Indeed, the enzyme-linked boronate immunoassay affinity (ELBIA) method for determining GA reported by Ikeda et al. (1998) generated higher values as well.

Boronate affinity chromatography methodology stands in contrast to assays using monoclonal antibodies, which are monospecific to each molecule. Low GA percentages in Table 1 are associated with assays based on monoclonal isolation, such as enzyme-linked immunosorbent assay (ELISA) and immunonephelemetry (turbidimetric) as well as charge dependent gel electrophoresis. Separation by HPLC and the newer Japanese enzymatic methodologies referred to above, which include amino acid elimination and both BCG and bromcresol purple dye quantification, result in the highest values. The Japanese enzymatic methodology is the basis for the new Lucica Ga-l Glycoalbumin assay kit. This kit, which measured GA percentages in the mid-twenties, was used in a recent report by Moriyama et al. (2005) regarding a hypothyroid female whose diabetes was undetected by traditional glucose and A1c monitoring. Kouzuma and colleagues have reported good correlation between the GA values generated by enzymatic technologies and those generated from HPLC. HPLC represents older technology to many investigators, and Cohen (1991) has reported that the associated high GA values are related to sample size and incomplete separation.

Conclusion

The Moriyama report indicates the value of a glycated albumin index and at the same time points to a lack of standardization of GA values. Current reference standardization of SMBG and A1c values is relatively acceptable, but substantial efficacy issues have been raised regarding a diabetes-monitoring paradigm based primarily on SMBG and A1c, especially for the millions of type 2 patients. A reliable index of intermediate glycation should lead to tighter diabetic control for these patients by providing additional information on the daily and weekly glucose fluctuations which have recently been linked to the deleterious effects of free radical molecule superoxide formation (Monier et al., 2006). Given the narrow normal range for GA% established at Quest with affinity chromatography, a reliable GA index appears likely to emerge from that separation technology. In addition, assays based on monoclonal isolation present a variety of reliable quantification stages, including ELISA, turbidimetric (ARUP), radiometric, and fluorescence. A promising diabetes monitoring standard is on the horizon, which now requires the concerted technological attention recently given to A1c.

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Table 1: Glycated Albumin Ranges for Normal and Diabetic Subjects From Scientific Literature

METHODOLOGY	DATE	NORMAL RANGE	MG/ML	DIABETIC RANGE	MG/ML	SOURCE	COMMENT
Affinity Chromatography +RIA	1985	0.43-1.19% (n=34)		0.68-5.00% (n=33)		Woo et al.	
Gel Electrophoresis+BCG	1987	0.4-2.0%	0.18-0.90	6.6-33.8%		Austin, Mullins, Morin	BCG criticised for lack of specificity
Affinity Chromotography on mAP boronate column	1991	0.6-1.8%		1.4-10.9%		Silver et al.	Criticized by Cruschetti et al.
Affinity Chromatography +immunoassay	1984	comparable		-		Conroy, Simon, Demetriou	
Immunonephelometry	1994	0.5-2.0%		-		Cabre et al.	
GlycoGel Test Kit	1992	1.40%	0.63	3.2-4.2% (type 1&2)		Bundschuh et al.	
Affinity Chromatography	1985	1.50%		5.15%	2.32	Rendell et al.	
Affinity Chromatography	1990	1.71% (n=11)		-		Ryle et al.	low and high fiber diets
Enzymatic Immunoassay	1990	2.0% (n=95)	0.9	2.9-5.1% (n=48)		Ardawi et al.	
Affinity Chromatography on boronate gels	1987	1.5-2.6%		1.9-7.3%		Ziel and Davidson	
Not Described	1997	-		5.4% (n=55 type 2)	2.43	Akens and Mays	Hassle effects on GA
Monoclonal Ab+ELISA	1989	2.40%	1.08	1.6-11.6%		Cohen and Hud	
Affinity Chromatography +immunoturbidometry	1986	2.30-6.30%		2.10-15.10%		Reed et al.	
ELBIA	1998	5.26% (n=110)	2.37	1.1-47.8%		Ikeda et al	
Ion Exchange Chromatography	1979	7.00%		-		Guthrow et al.	
Glycated Affinity Column +immunoturbidometry	1995	6.2-8.8%		12.1-12.9% (type 2)		Reaven et al.	
Glycated Affinity Column +BCG	1987	6.2-10.5% (n=20)	3.6	8-24% (n=20)		Ryan et al.	Gestational Diabetes
Not Described	1979	8.30%		-		Guthrow et al.	
Affinity Chromatography +Coomassie Blue	1984	8.60%	3.9	16.59%	7.5	Yatscoff et al.	
Affinity Chromatography on boronate gels	1983	10-12%	5	-		Garlick and Mazer	
HPLC	1990	16.1% (n=83)	7.2	39.1% (n=76)		Miyamoto et al.	
HP Affinity Chromatography column +boronate column	1992	16.10%		39.90%		Yasukawa et al.	
HPLC	1989	18%	8	-		Shima, Abe, Chikakiyo	
AA elim rx +Enzymatic+BCP	2004	17-<20%		>24%	>10.8	Kouzuma et al.	Basis for LUCICA GA-L
HPLC (Yamamoto, 1988)	1995	-		32% (n=9 Type 2)	14.4	Tahara and Shima	
HPLC	1991	20%	9	40%	18	Cohen	Criticism of HPLC
Enzymatic+BCG	2002	~40%		~80%		Kouzuma et al.	foreruuner of 2004 report