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SYMPOSIUM

The Clinical Use of Hemoglobin A1c

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Abstract

Hemoglobin A1c (HbA1c) has been accepted as an index of glycemic control since the mid-1970s and is the best marker for diabetic microvascular complications. Clinically, it is now used to assess glycemic control in people with diabetes. Assays are most reliable when certified by the National Hemoglobin Standardization Program but are subject to confounders and effect modifiers, particularly in the setting of hematologic abnormalities. Other measures of chronic glycemic control—fructosamine and 1,5-anhydroglucitol—are far less widely used. The relationship of HbA1c to average blood glucose was intensively studied recently, and it has been proposed that this conversion can be used to report an "estimated average glucose, eAG" in milligrams/deciliter or millimolar units rather than as per cent glycated hemoglobin. Finally, HbA1c has been proposed as a useful method of screening for and diagnosing diabetes.

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Introduction

The first description of hemoglobin A1c (HbA1c) has been variously ascribed to Kunkel[1] or Huisman,[2,3] but Rahbar, in 1969, is generally credited with the recognition of HbA1c as abnormal in diabetes.[4] He noted "an unusual hemoglobin" in diabetes. Bunn *et al.*[5] at Harvard, and Koenig *et al.*[6] at the Rockefeller University, established the identity of HbA1c as chemical glycation of N-terminal lysine and valines of hemoglobin A. The chemical reaction includes an initial, reversible, formation of the aldehyde Schiff base, followed by essentially irreversible Amadori rearrangement to the stable ketoamine.[5]

Early on, we evaluated HbA1c in diabetes at the General Clinical Research Center at Cornell University Medical College.[7] Before initiation of improved blood glucose control with insulin, the mean baseline fasting glycemia was 343 mg/dl, and the HbA1c was 9.8%. After 3 months of intensified diabetic control with insulin, the mean fasting blood glucose concentration was 84 mg/dl, and the HbA1c had dropped to 5.8%.

That report, in 1976, suggested that,

> Hemoglobin A1c concentration appears to reflect the mean blood sugar concentration best over previous weeks to months. The periodic monitoring of hemoglobin A1c levels provides a useful way of documenting the degree of control of glucose metabolism in diabetic patients and provides a means whereby the relation of carbohydrate control to the development of sequelae can be assessed.[7]

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Abbreviations: (ADAG) HbA1c Derived Average Glucose, (1,5-AG) 1,5-anhydroglucitol, (HbA1c) hemoglobin A1c, (CGM) continuous glucose monitoring, (DCCT) Diabetes Control and Complications Trial, (eAG) estimated average glucose, (NGSP) National Glycohemoglobin Standardization Program

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Indeed, the Diabetes Control and Complications Trial (DCCT) was designed over the subsequent 5 years, using HbA1c as the primary index of glycemia.[8] The DCCT and, subsequently, the United Kingdom Prospective Diabetes Study (UKPDS)[9] did, of course, establish once and for all the close relationship between glycemic control and the risk of diabetic complications.

Since those early years, HbA1c has not only become a standard part of diabetes care,[10] but a widely used tool in diabetes research. This discussion will review the clinical use of HbA1c, several other existing and potential approaches to assessing relatively long-term glycemia, the recently published reevaluation of the relationship between HbA1c and glycemia, the potential use of the new term "estimated average glucose" (eAG), and the potential for using HbA1c in the diagnosis of diabetes.

Current Use of HbA1c in Clinical Medicine

Hemoglobin A1c is the generally accepted best measure of glycemia over the prior 3 months. While there have always been, and continue to be, many ways to assess glycemia (e.g., history of overt symptoms [polyuria, polydypsia, etc.], urine glucose, random or fasting plasma glucose), HbA1c is unquestionably the best available. The occasional laboratory blood glucose may be the most frequently used of these assessment tools, and may be reasonably reflective of mean glycemia in stable type 2 diabetes, but it is a true measure only of blood glucose at that moment in time.

If HbA1c is within the target range, clinicians have a reliable indication that therapy is working appropriately and the risk of at least microvascular long-term complications is reduced.

The most reliable assays of HbA1c are, of course, those performed in a high quality clinical laboratory, one standardized to the National Glycohemoglobin Standardization Program (NGSP).[11] A number of point-of-care methods exist now that have also been certified by the NGSP. The major advantages of point-of-care testing include the fact that clinicians can know results immediately, as they see patients, rather than at some time after the visit, and the fact that point-of-care tests can be used at sites without easy access to clinical laboratories. The limitations of point-of-care testing include the need to have the reagents stored properly and the possible loss of quality control when untrained personnel perform the assay. Another limitation, which applies particularly to home testing of HbA1c by patients, is the fact that the data do not always accurately and completely enter into electronic medical records. Limitations aside, there is evidence that point-of-care testing is effective.[12,13]

Targets for HbA1c in Treating Diabetes

Targets for HbA1c in clinical practice are recommended by official organizations,[14,15] and these guidelines generally suggest either <6.5% or <7.0%, with a number of caveats. In fact, either of those levels of HbA1c do signal a low risk of developing progressive microvascular complications. There is only a minor difference in risk status between long-term control at the level of 6.5% or 7.0%, but the individualization of targets can make a considerable difference.

It has been suggested, for example, that in the elderly patient with multiple comorbid conditions, glycemic control has little benefit.[16,17] It also makes clinical sense to relax glycemic control for people with hypoglycemia unawareness or a history of severe hypoglycemia. A younger, more stable person with diabetes and good self-care, on the other hand, may be able to achieve even better glycemic control. We are believers in individualizing targets for HbA1c.

Clinical Confounders and Effect Modifiers

There are significant confounders and effect modifiers that influence HbA1c independent of glycemia.[10] The most common is anything that alters red blood cell survival. Since glycation of hemoglobin occurs only as the erythrocyte circulates in serum, hemoglobin in the older erythrocytes is more glycosylated, hemoglobin in the reticulocyte is less. Total HbA1c reflects the mix of older and younger erythrocytes. Therefore, if the average life of red cells is abnormally short (as in, for example, hemolytic anemia), then measured HbA1c will be lower, independent of glycemia. Conversely, if the average age of circulating erythrocytes is older (as, for example, occurs if erythropoiesis is suddenly stopped in an aplastic anemia), then the older red cell population would have higher HbA1c levels, regardless of glycemia.

Hemoglobinopathies, furthermore, can be confounded not only by altered red cell survival, but by abnormal hemoglobins (such as HbF), which can overlap in their electrophoretic peak with HbA1c, directly affecting the results of some assays. These complex assay-dependent interferences are well summarized on the NGSP website (http://www.ngsp.org/).

Among the controversial issues are four:

- Is there a glycemia-independent increase in HbA1c with aging? Some data suggest so.[18]
- Is there a glycemia-independent difference in HbA1c between ethnicities? There is evidence that non-Hispanic blacks in America have higher HbA1c for a given level of glycemia, whether diabetic[19] or pre-diabetic.[20]
- Are there "fast glycators" who have increased HbA1c for a given level of average glucose, and are these people more prone to diabetic complications.[21,22] Several studies suggest that both assertions are true, but the issue has been seriously disputed.[23,24]
- Finally, there is evidence that HbA1c is not a simple average of blood glucose over 3 months, but has a time-dependency, reflecting more closely the more recent levels of glycemia.[25,26]

Other Approaches to Assessing Long-term Glycemia

Other approaches to evaluating mean glycemia have been developed over the years. The assay of fructosamine is the most widely known.[27] In essence, fructosamine assays measure the glycation of all serum proteins. Since albumin accounts for most of the protein in blood, the fructosamine, for practical purposes, measures glycated albumin. As albumin has a turnover of about 2 weeks[26] (as compared to the 100–120 days of erythrocytes), fructosamine reflects glycemia over this far shorter period.

The fructosamine assay has been proposed for circumstances such as pregnancy, in which a short look at glycemia is desirable (although in the case of diabetic pregnancy, self-monitoring is more desirable since even a few weeks of hyperglycemia can be detrimental). When HbA1c is not valid, as in the circumstances described above, then fructosamine is a more useful alternative. On the whole, though, it has not been well standardized and is not widely used.[27,28]

Another newer approach is the measurement of 1,5 anhydroglucitol (1,5-AG), which is reabsorbed in the renal tubule, with competitive inhibition by glucose. Therefore, as glomerular filtration of glucose increases in poorly controlled diabetes, the tubular reabsorption of 1,5-AG decreases, urinary excretion of 1,5-AG increases, and serum levels of 1,5-AG fall.[29] Serum 1,5-AG therefore inversely reflects glucosuria, and a low 1,5-AG indicates more time spent with hyperglycemia. Interestingly, the assay has been promoted as an index of glucose lability,[30] since with more labile hyperglycemia, although the average glucose may not be higher than with stable blood glucose, the time spent inhibiting 1,5-AG would be increased, so serum 1,5-AG would be decreased.

HbA1c and the Average Blood Glucose

While HbA1c has been considered, since its development, a reflection of average blood glucose, the data describing that relationship were relatively sparse until 2008. The most widely used data were those gathered in the DCCT. The DCCT measured HbA1c quarterly, as well as a 7-point profiling of plasma glucose done by collecting capillary blood pre-meal, post-meal, and at bedtime.[31] They documented a linear relationship between mean plasma glucose and HbA1c. The regression line found HbA1c of 7% to 172 mg/dl and, rounded off, each 1% change in HbA1c reflected a change in mean plasma glucose of 35 mg/dl. This correlation was widely disseminated, and the conversion of HbA1c to mean plasma glucose was widely used. It was derived, however, from the very limited set of data taken only from people with type 1 diabetes in the DCCT.

Several years ago an international study, called "A1c Derived Average Glucose" (ADAG) was initiated to re-evaluate the relationship between HbA1c and average glucose.[32] The most obvious rationale for ADAG was to apply modern technology, especially continuous glucose monitoring (CGM), and far more frequent self-monitoring, to the question. The study also took place in a changing environment, when a new HbA1c "anchor"[33] was coming into effect, and new terms and new units were being heatedly discussed.[34,35]

The ADAG study, in 10 centers overall, had a rigorous glycemic monitoring protocol. Over a 3-month period, 507 subjects who completed the study self-monitored a mean of 5.1 times per day and had an average of 13 days of CGM.[36] Hemoglobin A1c was centrally measured for analysis at the end of the 3-month period. Of the participants, 53% had type 1 diabetes, 31% type 2 diabetes, and 16% did not have diabetes. While people from four countries participated, the racial diversity was not what had been hoped for, with 84% non-Hispanic white, 7% African or African American, 5% Hispanic, and 4% other. Adherence to the study protocol was excellent. There was also excellent concordance between the self-monitoring and the CGM data, suggesting that interstitial glucose levels were not systematically different from blood glucose.

The main result was notable. Hemoglobin A1c had a much tighter correlation with glucose than previously determined ($r = 0.92$ in ADAG[32] vs in $r = 0.82$ in the DCCT

data[31]). Furthermore, there was a different slope of the linear regression. The difference was clinically significant, for example redefining the glycemic correlate of HbA1c of 7.0% from 173 mg/dl[31] to 154 mg/dl, a finding consistent with an earlier, smaller study.[36]

The authors of the ADAG report went a step further, suggesting that since the average-glucose-to-HbA1c relationship had now been so closely defined, the term HbA1c should be replaced by the "estimated average glucose" (eAG). The proposal was quickly supported in an editorial from the American Diabetes Association.[37]

Should the eAG Replace the HbA1c?

The eAG would be expressed in conventional blood glucose units (mg/dl or mM), based entirely on the measured HbA1c. One appeal of the eAG is that the term HbA1c has always been difficult for professionals and patients alike to remember or to understand intuitively. Precedent exists in medicine for giving an "estimated" number based on other data, notably "eGFR," for estimated glomerular filtration rate, not measured but based on blood urea nitrogen and creatinine concentrations. But there are also downsides to converting our phrase from HbA1c to eAG.

To professionals using the term eAG, the various confounders and effect modifiers, and the remaining controversies surrounding non-glycemic influences on HbA1c could be largely forgotten if they simply read the result of the eAG. It would have to be understood that eAG is not in fact a direct measure of glucose, but a measure of glycated hemoglobin, affected by red cell survival, hemoglobinopathies, and so on.

For people with diabetes, there are also risks as well as possible benefits to changing terminology. A great deal of diabetes education, over many years, has taught the use of HbA1c, familiarized people with the term, and emphasized the importance of targets. Often, people with diabetes download or read out their own glucose monitor, noting an average of the self-tested results. This number could be quite different from the eAG, since the latter presumably reflects a 24-hour average rather than only an average of whatever times the patient self-monitored. The distinction between eAG and self tested result average, expressed in the same units (mg/dl), may be confusing. Hemoglobin A1c, expressed as a percent, is at least recognized as a different number.

The possible risks and benefits of changing terminology from HbA1c to eAG may be exaggerated. We have preliminary evidence suggesting that there is no difference in patient recall or understanding whether the term "estimated average glucose" or "A1c" is used (in press, Diabetes Educator). For the time being, the compromise between the international clinical chemistry groups and representatives of the diabetes professional community recommended that three terms be used by clinical laboratories when reporting the glycated hemoglobin results: HbA1c in conventional terms (%); eAG based on the ADAG study, in mg/dl or mM; and the term the chemists prefer, millimoles glycated per millimole hemoglobin.[38]

Use of HbA1c in Diagnosing Diabetes

The under-diagnosis of diabetes is most recently estimated as about 40%.[39] Whether directly causal or not related, the fact is that current criteria for diagnosing diabetes—mainly the fasting plasma glucose[14]—does not make it easy for the patient or the clinician.

Oral glucose tolerance tests are rarely done in clinical practice, at least in the United States, and the casual (non-fasting) plasma glucose over 200 mg/dl, with classic symptoms, is an extremely insensitive test. Therefore, if the patient arrives at a doctor's office in the non-fasted state, early diabetes will rarely be diagnosed.

The most obvious rationale for using HbA1c as a diagnostic test, then, is the fact that it can be done without fasting. People coming to their physician appointments at any time of day could be tested. Another prominent rationale is that HbA1c is not affected, as is fasting plasma glucose, by several days or a week of changed behavior, such as a low-calorie diet or vigorous exercise.

The reasons HbA1c was not accepted as a diagnostic criterion for diabetes by the American Diabetes Association's Expert Committee in 1997, were mainly the imprecision of the assay in various laboratories, and questions about sensitivity.[40] The NGSP has made enormous strides in enforcing comparability among commercial laboratories.[11] Issues of both sensitivity and specificity have also been addressed by data published in recent years.[41,42]

An unofficial panel was formed to review the literature, and published a consensus recommendation in 2008.[43] The panel agreed that the time had come to readdress the issue, and that the weight of evidence supports the use of HbA1c as a diagnostic test in establishing diabetes. Sensitivity and specificity of various cut-points were described based particularly on National Health and Nutrition Examination Survey data, but receiver operator

characteristic analysis found very acceptable sensitivity and specificity. This unofficial panel concluded that a confirmed HbA1c of 6.5% would yield a specificity of 99.6%, and a sensitivity of 42–44%. Thus 6.5% was recommended as the diagnostic threshold, and an HbA1c over 7% did not need glycemic value confirmation.[43]

Whatever cut-points or caveats are inserted, HbA1c will be recommended as an acceptable criterion for diagnosing diabetes. Translating recommendations into general acceptance, however, is an inexact, ill-defined process. The American Diabetes Association, the American Association of Clinical Endocrinology, and the European Association for the Study of Diabetes will weigh in with expert recommendations. The less defined phase involves the less specialized major professional organizations—such as the American College of Physicians, the American Medical Association, and the American Society of Preventive Medicine. It remains to be seen how quickly they, and other associations around the world, accept the concept of HbA1c as a diagnostic criterion for diabetes. But in fact, for years the clinical community has unofficially used HbA1c to screen for diabetes, so this may be a case of officialdom catching up with the practitioners.

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References:

1. Kunkel HG, Wallenius G. New hemoglobin in normal adult blood. Science. 1955;122(3163):288.
2. Huisman TH, Dozy AM. Studies on the heterogeneity of hemoglobin. V. Binding of hemoglobin with oxidized glutathione. J Lab Clin Med. 1962;60:302-19.
3. Kilpatrick ES. Haemoglobin A1c in the diagnosis and monitoring of diabetes mellitus. J Clin Pathol. 2008;61(9):977-82.
4. Rahbar S, Blumenfeld O, Ranney HM. Studies of an unusual hemoglobin in patients with diabetes mellitus. Biochem Biophys Res Commun. 1969:36(5):838-43.
5. Bunn HF, Haney DN, Kamin S, Gabbay KH, Gallop PM. The biosynthesis of human hemoglobin A1c: Slow glycosylation of hemoglobin in vivo. J Clin Invest. 1976;57(6):16529.
6. Koenig RJ, Blobstein SH, Cerami A. Structure of carbohydrate of hemogloban A1c. J. Biol. Chem. 1977:252(9):2992-2997.
7. Koenig RJ, Peterson CM, Jones RL, Saudek C, Lehrman M, Cerami A. Correlation of glucose regulation and hemoglobin A1c in diabetes mellitus. N Engl J Med. 1976;295(8):417-20.
8. 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(14):977-86.
9. UK Prospective Diabetes Study (UKPDS) Group. Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). Lancet. 1998;352(9131):854-65.
10. Saudek CD, Derr RL, Kalyani RR. Assessing glycemia in diabetes using self-monitoring blood glucose and hemoglobin A1c. JAMA. 2006;295(14):1688-97.
11. Little RR. Glycated hemoglobin standardization--National Glycohemoglobin Standardization Program (NGSP) perspective. Clin Chem Lab Med. 2003;41(9):1191-8.
12. Cagliero E, Levina EV, Nathan DM. Immediate feedback of HbA1c levels improves glycemic control in type 1 and insulin-treated type 2 diabetic patients. Diabetes Care. 1999;22(11):1785-9.
13. Miller CD, Barnes CS, Phillips LS, Ziemer DC, Gallina DL, Cook CB, Maryman SD, El-Kebbi IM. Rapid A1c availability improves clinical decision-making in an urban primary care clinic. Diabetes Care. 2003;26(4):1158-1163.
14. American Diabetes Association. Standards of medical care in diabetes--2009. Diabetes Care. 2009;32(Suppl 1):S13-61.
15. Rodbard HW, Blonde L, Braithwaite SS, Brett EM, Cobin RH, Handelsman Y, Hellman R, Jellinger PS, Jovanovic LG, Levy P, Mechanick JI, Zangeneh F, AACE Diabetes Mellitus Clinical Practice Guidelines Task Force. American Association of Clinical Endocrinologists medical guidelines for clinical practice for the management of diabetes mellitus. Endocr Pract. 2007;13(Suppl 1):1-68.
16. Huang ES, Zhang Q, Gandra N, Chin MH, Meltzer DO. The effect of comorbid illness and functional status on the expected benefits of intensive glucose control in older patients with type 2 diabetes: A decision analysis. Ann Intern Med. 2008;149(1):11-9.
17. Saudek CD, Kalyani RR. Does comorbidity affect the benefit of intensive glycemic control in older patients with type 2 diabetes mellitus? Nat Clin Pract Endocrinol Metab. 2009;5(2):78-9.
18. Pani LN, Korenda L, Meigs JB, Driver C, Chamany S, Fox CS, Sullivan L, D'Agostino RB, Nathan DM. Effect of aging on A1C levels in individuals without diabetes: Evidence from the Framingham Offspring Study and the National Health and Nutrition Examination Survey 2001-2004. Diabetes Care. 2008;31(10):1991-6.
19. Saaddine JB, Fagot-Campagna A, Rolka D, Narayan KM, Geiss L, Eberhardt M, Flegal KM. Distribution of HbA(1c) levels for children and young adults in the U.S.: Third National Health and Nutrition Examination Survey. Diabetes Care. 2002;25(8):1326-30.
20. Herman WH, Ma Y, Uwaifo G, Haffner S, Kahn SE, Horton ES, Lachin JM, Montez MG, Brenneman T, Barrett-Connor E, Diabetes Prevention Program Research Group. Differences in A1C by ace and ethnicity among patients with impaired glucose tolerance in the Diabetes Prevention Program. Diabetes Care. 2007;30(10):2453-7.
21. Hempe JM, Gomez R, McCarter RJ, Jr., Chalew SA. High and low hemoglobin glycation phenotypes in type 1 diabetes: A challenge for interpretation of glycemic control. J Diabetes Complications. 2002;16(5):313-20.
22. McCarter RJ, Hempe JM, Gomez R, Chalew SA. Biological variation in HbA1c predicts risk of retinopathy and nephropathy in type 1 diabetes. Diabetes Care. 2004;27(6):1259-64.
23. Genuth S, Lachin JM, Nathan DM. Biological variation in HbA1c predicts risk of retinopathy and nephropathy in type 1 diabetes: Response to McCarter et al. Diabetes Care. 2005;28(1):233; author reply 234-5.

24. Rohlfing C, Wiedmeyer HM, Little R, Grotz VL, Tennill A, England J, Madsen R, Goldstein D. Biological variation of glycohemoglobin. Clin Chem. 2002;48(7):1116-8.

25. Beach KW. A theoretical model to predict the behavior of glycosylated hemoglobin levels. J Theor Biol. 1979;81(3):547-61.

26. Tahara Y, Shima K. Kinetics of HbA1c, glycated albumin, and fructosamine and analysis of their weight functions against preceding plasma glucose level. Diabetes Care. 1995;18(4):440-7.

27. Lindsey CC, Carter AW, Mangum S, Greene D, Richardson A, Brown SJ, Essary JL, McCandless B. A prospective, randomized, multicentered controlled trial to compare the annual glycemic and quality outcomes of patients with diabetes mellitus monitored with weekly fructosamine testing versus usual care. Diabetes Technol Ther. 2004;6(3):370-7.

28. Narbonne H, Renacco E, Pradel V, Portugal H, Vialettes B. Can fructosamine be a surrogate for HbA(1c) in evaluating the achievement of therapeutic goals in diabetes? Diabetes Metab. 2001;27(5 Pt 1):598-603.

29. Buse JB, Freeman JL, Edelman SV, Jovanovic L, McGill JB. Serum 1,5-anhydroglucitol (GlycoMark): A short-term glycemic marker. Diabetes Technol Ther. 2003;5(3):355-63.

30. McGill JB, Cole TG, Nowatzke W, Houghton S, Ammirati EB, Gautille T, Sarno MJ, U.S. trial of the GlycoMark assay. Circulating 1,5-anhydroglucitol levels in adult patients with diabetes reflect longitudinal changes of glycemia: A U.S. trial of the GlycoMark assay. Diabetes Care. 2004;27(8):1859-65.

31. Rohlfing CL, Wiedmeyer HM, Little RR, England JD, Tennill A, Goldstein DE. Defining the relationship between plasma glucose and HbA(1c): Analysis of glucose profiles and HbA(1c) in the Diabetes Control and Complications Trial. Diabetes Care. 2002;25(2):275-8.

32. Nathan DM, Kuenen J, Borg R, Zheng H, Schoenfeld D, Heine RJ, A1c-Derived Average Glucose (ADAG) Study Group. Translating the A1C assay into estimated average glucose values. Diabetes Care. 2008;31(8):1473-8.

33. Jeppsson JO, Kobold U, Barr J, Finke A, Hoelzel W, Hoshino T, Miedema K, Mosca A, Mauri P, Paroni R, Thienpont L, Umemoto M, Weykamp C, International Federation of Clinical Chemistry and Laboratory Medicine (IFCC). Approved IFCC reference method for the measurement of HbA1c in human blood. Clin Chem Lab Med. 2002;40(1):78-89.

34. Hoelzel W, Weykamp C, Jeppsson JO, Miedema K, Barr JR, Goodall I, Hoshino T, John WG, Kobold U, Little R, Mosca A, Mauri P, Paroni R, Susanto F, Takei I, Thienpont L, Umemoto M, Wiedmeyer HM, IFCC Working Group on HbA1c Standardization. IFCC reference system for measurement of hemoglobin A1c in human blood and the national standardization schemes in the United States, Japan, and Sweden: A method-comparison study. Clin Chem. 2004;50(1):166-74.

35. Berg AH, Sacks DB. Haemoglobin A1c analysis in the management of patients with diabetes: From chaos to harmony. J Clin Pathol. 2008;61(9):983-7.

36. Nathan DM, Turgeon H, Regan S. Relationship between glycated haemoglobin levels and mean glucose levels over time. Diabetologia. 2007;50(11):2239-44.

37. Kahn R, Fonseca V. Translating the A1C assay. Diabetes Care. 2008;31(8):1704-7.

38. Consensus Committee. Consensus statement on the worldwide standardization of the hemoglobin A1C measurement: The American Diabetes Association, European Association for the Study of Diabetes, International Federation of Clinical Chemistry and Laboratory Medicine, and the International Diabetes Federation. Diabetes Care. 2007;30(9):2399-400.

39. Cowie CC, Rust KF, Ford ES, Eberhardt MS, Byrd-Holt DD, Li C, Williams DE, Gregg EW, Bainbridge KE, Saydah SH, Geiss LS. Full accounting of diabetes and pre-diabetes in the U.S. population in 1988-1994 and 2005-2006. Diabetes Care. 2009;32(2):287-94.

40. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care. 1997;20(7):1183-97.

41. Tabaei BP, Herman WH. A multivariate logistic regression equation to screen for diabetes: Development and validation. Diabetes Care. 2002;25(11):1999-2003.

42. Johnson SL, Tabaei BP, Herman WH. The efficacy and cost of alternative strategies for systematic screening for type 2 diabetes in the U.S. population 45-74 years of age. Diabetes Care. 205;28(2):307-11.

43. Saudek CD, Herman WH, Sacks DB, Bergenstal RM, Edelman D, Davidson MB. A new look at screening and diagnosing diabetes mellitus. J Clin Endocrinol Metab. 2008;93(7):2447-53.