The Role of HbA1C Testing in Diagnosing Diabetes

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1. Overview of New Diagnostic Criteria of Diabetes – HbA1C

2. The role of HbA1C testing in diagnosing diabetes in Korean adults
A Typical Patient Encounter

“So, Mrs. Lee, it looks like you do have diabetes. Your random blood sugar was 195 mg/dl, but you have to perform fasting blood glucose or oral glucose tolerance test to diagnose the diabetes.”
Diagnosis of Diabetes

Based on long term complication

1997 Expert committee

- HbA1C ≥ 6.5%

1979 NDDG

- FPG ≥ 140 mg/dl
- 2hPG ≥ 200 mg/dl

Based on distribution of glucose levels

IGT: FPG < 140 mg/dl + 140 ≤ 2hPG < 200 mg/dl

FPG cut point ≥ 126 mg/dl
FPG: preferred test

Increased risk for diabetes: HbA1C 5.7-6.4%

FPG: Preferred test

2hPG ≥ 200 mg/dl

IFG: FBS ≥ 110 mg/dl (2003. 100mg/dl)
• 1997 expert committee report
  - against using A1C values for diagnosis
  - because of the lack of assay standardization

• 2003 follow-up report
  - A1C not be used to diagnose diabetes

• “What has changed” → “continued and further standardization of the A1C assay”
CAN THE HBA1C BE USED TO DIAGNOSE DIABETES?
Longterm complication

- Laboratory measures that capture long-term glycemic exposure: better marker of the disease than single measures of glucose concentration.

- Strong correlation between retinopathy and A1C but a less consistent relationship with fasting glucose level.
Relationship of retinopathy and FPG

Relationship of retinopathy and HbA1C

Relationship between HbA1c and any retinopathy (black), mild retinopathy (grey) and moderate retinopathy (white)

Sabanayagam C, Diabetologia 2009
ROC curves for FPG and Prevalent Retinopathy

(A: Blue Mountains Eye Study, B: The AusDiab Study
C: The MESA Study)

ROC curves for HbA1c (%) and the various microvascular complications.

Moderate retinopathy (AUC 0.904)
Mild retinopathy (AUC 0.899)
Any retinopathy (AUC 0.754)
Micro/macroalbuminuria (AUC 0.673)
Chronic kidney disease (AUC 0.615)
Peripheral neuropathy (AUC 0.573)

Sabanayagam C, Diabetologia 2009
Accuracy

• laboratory measurements of glucose and A1C: accuracy and precision of A1C assays at least match those of glucose assays.

• Biological variability of A1C within an individual is somewhat smaller than that of fasting glucose (CV 3.6 vs. 5.7%) and considerably less than that of 2-h glucose (CV 16.6%) – suggesting that repeated measurements would be more consistent using A1C.
The measurement of glucose itself is less accurate and precise than most clinicians realize!
- 41% of instruments have a significant bias from the reference method that would result in potential misclassification of > 12% of patients.
Lability of glucose vs. Relative stable HbA1C values

- Variability of HbA1C: less than that of FPG
day-to-day within-person variance of <2% for HbA1C but 12-15% for FPG.
- Potential preanalytic errors owing to sample handling and lability of glucose in the collection tube at room temperature.
- Convenience for the patient and ease of sample collection for A1C
Advantages of A1C testing compared with FPG or 2hPG for the diagnosis of diabetes

- Better index of overall glycemic exposure and risk for long-term complications
- Substantially less biologic variability
- Substantially less preanalytic instability
- No need for fasting or timed samples
- Relatively unaffected by acute (e.g. stress or illness related) perturbations in glucose levels
- Currently used to guide management and adjust therapy
WHAT IS THE MOST APPROPRIATE A1C CUT POINT FOR THE DIAGNOSIS OF DIABETES?
• HbA1C as a screening tool for detection of Type 2 diabetes: a systematic review: 6.1% (*Diabet Med. 2007 24:333-43*)


• Prevalence and prediction of unrecognised diabetes mellitus and impaired glucose tolerance following acute stroke: 6.2% (*Age Ageing. 2004 33:71-7*)

• HbA1c measurement improves the detection of type 2 diabetes in high-risk individuals with nondiagnostic levels of fasting plasma glucose: the Early Diabetes Intervention Program (EDIP): 6.1% (*Diabetes Care. 2001 24:465-71*)
'97 committee report: prevalence of retinopathy increase substantially at A1C between 6.0 and 7.0%.

DETECT-2 + ‘97 report (~28,000 subjects from 9 countries) the prevalence of “moderate retinopathy” begins to rise at 6.5%
• In selecting a diagnostic A1C level ≥ 6.5%, the international Expert Committee balanced the stigma and costs of mistakenly identifying individuals as diabetic against the minimal clinical consequences of delaying the diagnosis in someone with an A1C level < 6.5%.

• Emphasize specificity rather than sensitivity.
LIMITATIONS OF A1C AS THE RECOMMENDED MEANS OF DIAGNOSING DIABETES
Some hemoglobin traits (HbS, HbC, HbF, and HbE) currently may be corrected for by many PASS methods. Affinity PASSays that are unaffected by hemoglobin traits may be used.

<table>
<thead>
<tr>
<th>Method</th>
<th>Interference from</th>
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<td>HbAS</td>
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<tr>
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</tr>
<tr>
<td>Axis-Shield Afinion</td>
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<tr>
<td>Bayer (Metrika) A1cNOW</td>
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<tr>
<td>Beckman Synchron System</td>
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<td>Bio-Rad D-10</td>
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<td>Bio-Rad Variant A1c</td>
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<td>Bio-Rad Variant II Turbo A1c</td>
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<td>Dade Dimension</td>
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<td>Olympus AU system</td>
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<td>Ortho-Clinical Vitros</td>
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<td>Primus HPLC (affinity)</td>
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<tr>
<td>Roche Cobas Integra Gen.2</td>
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<td>Roche/Hitachi (Tina Quant II)</td>
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<tr>
<td>Siemens (Bayer) Advia HbA1c* (original version)</td>
<td>Yes</td>
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<td>Siemens (Bayer) Advia A1c (new version)</td>
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<td>Siemens (Bayer) DCA 2000</td>
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<td>Tosoh G7</td>
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<tr>
<td>Tosoh G8</td>
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</table>
Any Condition that Changes Red Cell Turnover

- hemolytic anemia
- chronic malaria
- major blood loss
- blood transfusions
Age

A1C levels appear to increase with age, but the extent of the change, whether it relates to factors other than glucose metabolism, and the effect of the age-related increases on the development of complications are not sufficiently clear to adopt age-specific values in a diagnostic scheme.
Effect of **Aging** on HbA1C levels in Individuals without Diabetes

![Graphs showing the effect of aging on HbA1C levels in individuals without diabetes.](DiabetesCare_2008_31_1991-6.png)

*Diabetes Care. 2008, 31:1991-6*
Race

- racial disparities in A1C: premature to establish race-specific diagnostic values
- multivariate analysis of 15,934 nondiabetic participants in the 1999-2006 NHANES,
  - non hispanic blacks had 2.4 fold increase in likelihood of A1C > 6% among subjects with fasting glucose< 100mg/dl.
- subjects with IGT in the Diabetes Prevention Program, mean A1C was 5.78% for whites and 6.18% for blacks.
Other conditions

- rapidly evolving type 1 diabetes: diabetes should be diagnosable with typical symptoms and casual glucose levels $\geq 200$ mg/dl

- Iron deficiency anemia, effects of HIV therapy, renal failure, dapsone therapy, high dose salicylates, vitamin C, E, splenectomy and aplastic anemia
Iron deficiency anemia

: increase in HbA1C by 1-1.5%
that subsequently falls following iron treatment.

*Diabet Med. 2007 24:843-7*
Discrepancies between HbA1C and glucose levels

• HbA1C represents glycation of hemoglobin, localized to a specific biologic compartment, the erythrocyte cytoplasm, which is potentially rather different from the entire glucose distribution volume.

• Erythrocyte turnover, cell membrane permeability to glucose, hemoglobin glycation and deglycation, and a myriad of other processes will change glycated hemoglobin levels.
Pregnancy

- reduction in HbA1C levels, perhaps as a function of hemodilution or increased erythrocyte turnover

- during late pregnancy, A1C levels decrease by \(~0.5\%\) at every level of mean plasma glucose.

*Diabetes Care.* 2007, 30:1579-80
Underdiagnosis v. Overdiagnosis

NHANES data

50-60% of patients with fasting plasma glucose ≥126 mg/dl had HbA1C < 6.5%

- suggesting that HbA1C might reduce the number of people diagnosed as having diabetes from that using current glycemic criteria.
HbA1C will lead to overdiagnosis among the elderly, blacks, subject with iron deficiency, and individuals genetically predisposed to greater levels of hemoglobin glycation, whereas those with anemia, renal insufficiency, and many hemoglobinopathies, as well as those with other genetic variations, will be incorrectly told that they do not have diabetes.
Practical Issues related to A1C Testing

• Testing be performed in a laboratory using a method that is NGSP certified, POC (point of care) instruments have not yet been shown to be sufficiently accurate or precise for diagnosing diabetes

• POC devices:
  - biases ranged from approximately 0.9 to 0.4%.

• No POC device for measuring HbA1C be used for the diagnosis of diabetes.
Distribution of estimated numbers of persons without a history of diabetes in the US 2000 Census population (age $\geq$20 years) at different HbA1C cutpoints

Criteria for the diagnosis of diabetes
2010 ADA Recommendation

1. A1C≥6.5%. The test should be performed in a laboratory using a method that is NGSP certified and standardized to the DCCT assay
   OR
2. FPG≥126 mg/dl. Fasting is defined as no caloric intake for at least 8h.
   OR
3. 2-h plasma glucose≥200 mg/dl during an OGGT. The test should be performed as described by the World Health Organization, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water
   OR
4. In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose≥200 mg/dl

HbA1C cut point of 6.1% has an optimal sensitivity and specificity and can be used as a screening test, and a cut point of 6.5% has optimal specificity of 88% for diagnosis of diabetes.

(J Clin Endocrinol Metab 2010, e-published)
An A1C level of $\geq 5.8\%$ had highest combination of sensitivity (72%) and specificity (91%) for identifying newly diagnosed diabetes.
The A1C cut point of **6.15%** yielded the highest combination of sensitivity (63%) and specificity (60%).
Glycated Hemoglobin, Diabetes, and Cardiovascular Risk in Nondiabetic Adults

HbA1C was superior to fasting glucose for assessment of the long-term risk of subsequent cardiovascular disease, especially at values above 6.0%

NEJM 2010;362:800-11
Brief report
New diagnosis criteria for diabetes with hemoglobin A1c and risks of macro-vascular complications in an urban Japanese cohort: The Suita Study

Incident rates and adjusted HRs with 95% CIs for cardiovascular diseases by HbA1c levels in a cohort study of the Japanese men and women, 1989–2005.

<table>
<thead>
<tr>
<th>HbA1c levels</th>
<th>N</th>
<th>Number of events</th>
<th>Person-years</th>
<th>Crude incidence rates (per 1000 person-years)</th>
<th>Age-adjusted</th>
<th>Multivariate-adjusteda</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HRs</td>
<td>95% CIs</td>
<td>HRs</td>
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<tr>
<td>≤5.9</td>
<td>1451</td>
<td>54</td>
<td>18627</td>
<td>2.9</td>
<td>1</td>
<td>(reference)</td>
</tr>
<tr>
<td>6.0–6.4</td>
<td>108</td>
<td>9</td>
<td>1289</td>
<td>7.0</td>
<td>1.5</td>
<td>(0.7–3.0)</td>
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<tr>
<td>&gt;0.5</td>
<td>40</td>
<td>7</td>
<td>479</td>
<td>14.8</td>
<td>0.5</td>
<td>(1.0–7.7)</td>
</tr>
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</table>

Trend P = 0.003
Trend P = 0.04

(Diabetes Res and Clin Pract, 2010)
THE ROLE OF HBA1C TESTING IN DIAGNOSING DIABETES IN KOREAN ADULTS
Subject

• Recruited 996 adults

(mean age 41 ± 14 years, mean BMI 23.1 ± 3.5 kg/m²)
without a self-reported history of diabetes
from 8 university hospitals in 2009
Method

- 75-g OGTT and HbA1C sampling were performed in all examinees.
- Glucose concentrations were measured by colorimetry method (ADVIA2400 autoanalyzer)
  HbA1C, by immunoturbidimetric method (Cobas integra800, Roche, Switz)
- at the central laboratory.
- Receiver operating characteristic curve analysis was used to examine the sensitivity and specificity of HbA1C for diagnosing diabetes.
# Clinical characteristics of subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>41 ± 14</td>
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<tr>
<td>Sex (male/female)</td>
<td>203/ 793</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.1 ± 3.5</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>115 ± 14</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>73 ± 9</td>
</tr>
<tr>
<td>Fasting glucose (mg/dl)</td>
<td>95 ± 21</td>
</tr>
<tr>
<td>Post 2hr glucose (mg/dl)</td>
<td>125 ± 58</td>
</tr>
<tr>
<td>HbA1C (%)</td>
<td>5.9 ± 0.6</td>
</tr>
<tr>
<td>Family history of diabetes (yes/no)</td>
<td>237/ 759</td>
</tr>
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</table>
Scatter plots of FPG and 2h postload glucose in relation to A1C

$r=0.782, p<0.01$

$r=0.726, p<0.01$
ROC curve for identification of participants with previously undiagnosed diabetes, using HbA1C for diagnosis and an OGTT as criterion.

<table>
<thead>
<tr>
<th></th>
<th>AUC</th>
<th>95% CI</th>
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</thead>
<tbody>
<tr>
<td>HbA1C</td>
<td>0.911</td>
<td>0.880-0.942</td>
</tr>
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</table>
ROC curve for identification of participants with previously undiagnosed IGR, using HbA1C for diagnosis and an OGTT as criterion.

<table>
<thead>
<tr>
<th>AUC</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA1C</td>
<td>0.825</td>
</tr>
<tr>
<td></td>
<td>0.794-0.856</td>
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</table>
ROC curve analysis for HbA1C according to sex

<table>
<thead>
<tr>
<th></th>
<th>AUC</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA1C</td>
<td>0.914</td>
<td>0.874-0.953</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>HbA1C</td>
<td>0.888</td>
</tr>
</tbody>
</table>
Cutoff value of HbA1C according to age

![Graph showing the cutoff value of HbA1C according to age categories.]

- Age (yrs): 21~30, 31~40, 41~50, 51~60, 61~
- HbA1C (%): 5.9, 6.1, 6.1, 6.2, 6.3
### Mean HbA1C by age categories in subjects with NGT

<table>
<thead>
<tr>
<th>Age (n)</th>
<th>HbA1C (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>21~30 (288)</td>
<td>5.55 ± 0.21</td>
</tr>
<tr>
<td>31~40 (269)</td>
<td>5.63 ± 0.24</td>
</tr>
<tr>
<td>41~50 (139)</td>
<td>5.69 ± 0.29</td>
</tr>
<tr>
<td>51~60 (170)</td>
<td>5.73 ± 0.29</td>
</tr>
<tr>
<td>61~ (122)</td>
<td>5.84 ± 0.36</td>
</tr>
</tbody>
</table>

![Graph showing the trend of HbA1C with age](image)
Sensitivity and specificity of HbA1C 6.1%, 6.5% as cut-off points for diagnosing the diabetes

<table>
<thead>
<tr>
<th>Cut-off point</th>
<th>PPV</th>
<th>NPV</th>
<th>sensitivity</th>
<th>specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.1%</td>
<td>36.1%</td>
<td>97.9%</td>
<td>84.8%</td>
<td>82.3%</td>
</tr>
<tr>
<td>6.5%</td>
<td>68.1%</td>
<td>96.0%</td>
<td>59.0%</td>
<td>97.5%</td>
</tr>
</tbody>
</table>

PPV, positive predictive value; NPV, negative predictive value
HbA1C (6.5%) based DM: 9.1% 
OGTT based DM: 10.5% 

68.1% had diabetic glucose levels 
Total 996
HbA1C (6.1%) based DM: 24.8%
OGTT based DM: 10.5%

36.0% had diabetic glucose levels

Total 996
The cutoff point for diagnosing diabetes with the highest sum of sensitivity and specificity in our data was an HbA1C level of 6.1%.

HbA1C levels positively associated with age, but results in sex-stratified analysis were similar.

Of all subjects with an HbA1C > 6.1%, 36% had diabetic glucose levels.

HbA1C at 6.1% provided high sensitivity (84.8%) and high NPP (97.9%), while HbA1C at 6.5% gave high specificity (97.5%) and high PPV (68.1%).
Conclusion

Further studies should be undertaken to determine

- the population-specific HbA1C cut-offs points

- whether the increase in HbA1C associated with age is of clinical significance and to clarify whether age-specific diagnostic and treatment criteria would be appropriate.
Acknowledgement

• Yeon-Ah Sung, Ewha Womans University
• Choon Hee Chung, Yonsei University
• Dong-Jun Kim, Inje University
• Eun-Jung Rhee, Sungkyunkwan University
• Ie Byung Park, Gachon University
• Jee-Young Oh, Ewha Womans University
• Sin Gon Kim, Korea University
• Sungdae Moon, Catholic University
• Sung-Hoon Kim, Kwandong University
Thank You!