Laboratory Diagnosis & Monitoring of Diabetes Mellitus

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Objectives

• Explain the rationale for using HbA$_{1c}$ to diagnose diabetes mellitus
• List the components of a HbA$_{1c}$ result report based on international consensus
• Be able to describe how the estimated average glucose is calculated and the limitations of its use
Outline

• Overview of diabetes mellitus
• Clinical significance of HbA$_{1c}$
• Laboratory methods for HbA$_{1c}$
• HbA$_{1c}$ and mean plasma glucose
• Effects of variant hemoglobins on HbA$_{1c}$
• Appropriate laboratory testing
• Conclusions
Statistics

- Afflicts 24 million Americans
- Approximately 6-7 million are undiagnosed
- 5-10% Type 1 (juvenile)
  - Insulin deficiency
- 90-95% Type 2 (adult)
  - Insulin resistance
  - Relative insulin deficiency
Diabetic Complications

• Leading cause of:
  – Kidney failure
  – Adult blindness
  – Non-traumatic amputations

• People with diabetes are 2-4 times more likely to have an MI or CVA

• Single most costly chronic disease

• Sixth leading cause of death in US
Non-enzymatic Glycation of Hb

- **HbA$_1$:** glycated Hb A (80% is HbA$_{1c}$)
- **HbA$_{1c}$:** glucose at N-terminus of β chain
- **HbA$_{1a}$:** fructose-1,6-diphosphate (HbA$_{1a1}$) or glucose-6-phosphate (HbA$_{1a2}$) at N-terminus of β chain
- **HbA$_{1b}$:** pyruvate at N-terminus of β chain
- **HbA** can be glycated on lysine residues
Glycation of Hb

N-terminal amino group Glucose

$\text{NH}_2$ Glucose $\beta A$

Aldimine (Schiff base)

$\text{H} - \text{C} = \text{N} - \beta A$

Ketoamine

$\text{H}_2\text{C} - \text{NH} - \beta A$

$\text{H} - \text{C} = \text{O}$

$\text{C} = \text{O}$

Hb A + Glucose $\xleftrightarrow{\text{rapid}}$ pre-Hb A$_{1c}$ $\xrightarrow{\text{slow}}$ Hb A$_{1c}$

**FIGURE 24-15.** Formation of hemoglobin A$_{1c}$. 

Tietz Textbook of Clinical Chemistry 3rd edition, 1999
Clinical Utility of HbA$_{1c}$

- It reflects mean plasma glucose over the preceding 6-12 weeks
- Mean plasma glucose of preceding 1 month accounts for about 50% of HbA$_{1c}$
- It is the only marker that correlates well with long term complications
- Glycation of serum proteins reflects mean plasma glucose over the preceding 2-3 weeks
ADA Guidelines for Diagnosis

- Test people over 45 y.o. every 3 years
- Overweight or family history test earlier
- $\text{HbA}_{1c} > 6.4\%$ (new in 2009)
- Fasting plasma glucose $> 125 \text{ mg/dL}$
- Symptoms plus random glucose $> 199 \text{ mg/dL}$
- 2 hr post glucose challenge $> 199 \text{ mg/dL}$ (75g glucose load)
Retinopathy and FPG, 2hPG, HbA$_{1c}$

Diabetes Care 2009;32:1-8
Retinopathy and HbA$_{1c}$

The International Expert Committee, Diabetes Care 2009;32:1-8
Advantages of HbA$_{1c}$ for Diagnosis

- Standardized and aligned to DCCT and UKPDS
- Better index of overall glycemic exposure and risk for long-term complications
- Less biologic variability than glucose
- Less preanalytic variability than glucose
- No need for fasting or timed samples
- Relatively unaffected by acute perturbations of glucose concentrations (e.g. stress or illness)
- Currently used to guide management and adjust therapy
Abnormal Glucose Homeostasis

- Metabolic stage intermediated between normal glucose homeostasis and diabetes (nearly normal HbA\textsubscript{1c})
- Impaired fasting glucose (IFG)
  - Fasting plasma glucose 100-125 mg/dL
- Impaired glucose tolerance (IGT)
  - 2 hr post glucose challenge 140-199 mg/dL (75g glucose load)
- HbA\textsubscript{1c} 6.1-6.4\%
General Limitations of HbA$_{1c}$

- **False increases**
  - Iron deficiency
    - Non-diabetics subjects with severe iron deficiency had HbA$_{1c}$ results up to 7.5%
  - Asplenia leads to increased RBC survival and increased HbA$_{1c}$
  - Analytical—Hb variants can comigrate with HbA$_{1c}$

- **False decreases**
  - Shortened RBC survival
    - Unstable hemoglobins
    - Autoimmune hemolytic anemias
  - Analytical
    - Hemoglobinopathies
DCCT

- Diabetes Control and Complications Trial
- Type 1 diabetic patients
- Two groups
  - Conventional management
  - Intensive management
    - Goal normalization of blood glucose
    - Goal not achieved
- Seven year average study period
DCCT Results

• Conventional group
  – Mean plasma glucose ~210 mg/dL
  – HbA$_{1c}$ 9%

• Intensive treatment group
  – Mean plasma glucose ~160 mg/dL
  – HbA$_{1c}$ 7.2%

• Non-diabetic
  – Mean plasma glucose ~100 mg/dL
  – HbA$_{1c}$ <6%
Intensive Treatment Group

• Showed a 60% reduction in retinopathy, nephropathy, and neuropathy
• Threefold greater risk of hypoglycemia
• Increased costs of intensive control offset by decreased complications and more productive lives
UKPDS

- United Kingdom Prospective Diabetes Study
- Type 2 diabetic patients
- Followed an average of 10 years
- Conventional group HbA$_{1c}$ 7.9%
- Intensive group HbA$_{1c}$ 7.0%
- Microvascular complications reduced 25%
- Hyperglycemia causes these complications
- Intensive therapy may reduce CAD
Benefits of Nearly Normal HbA$_{1c}$

- Five extra years of life
- Delays progression of eye, kidney, and nerve disease
  - Eight additional years of sight
  - Six extra years free from kidney disease
- Increased work productivity
- Lower health care use
ADA Guidelines for Monitoring

- HbA$_{1c}$ <5.7% is normal
- HbA$_{1c}$ <7% meets goal
- HbA$_{1c}$ 7-8%
- HbA$_{1c}$ >8% additional action suggested

- AACE and ACE goal for HbA$_{1c}$ is <6.5%
HbA$_{1c}$ Methods

- Cation exchange chromatography
  - Measures HbA$_{1c}$ as percent of total Hb A
- Boronate affinity chromatography
  - Measures glycated Hb as percent of total Hb
- Immunoassay
  - Measures glycated N-terminus of the $\beta$-chain as percent of total Hb
- Enzymatic
  - Measures glycated N-terminus of the $\beta$-chain as percent of total Hb
- POCT vs. central laboratory
CAP PT Surveys

• Fresh whole blood PT samples
  – NGSP assigned values
  – Minimal matrix effects
  – Permit assessment of method performance in the field
  – Provide imprecision information across reagent lots and analyzers
• Performance has improved since introduction
• Accuracy based grading of +/- 8% of NGSP value
POCT for HbA$_{1c}$

- Atlanta study
  - Favorable impact of POCT on F/U HbA$_{1c}$
  - Enhanced patient and/or provider motivation

- Boston study
  - Decrease in HbA1c in POC group but not central lab group at 6 and 12 months

- UK study
  - No difference in proportion of patients with HbA$_{1c} <$7%
  - No difference in total cost

- Australian study sponsored by Australian DoH
  - POCT provided slightly better clinical effectiveness
  - POCT considerably more expensive than central lab alternative
Reporting Results

- ADA, EASD, IFCC, IDF
- Results for HbA$_{1c}$ are to be reported worldwide
  - Mmol/mol (IFCC reference method equivalent)
  - % (NGSP equivalent, current reporting unit)
  - Estimated average glucose (eAG) which corresponds to average plasma glucose
- Worldwide study provided an equation to transform HbA$_{1c}$ into eAG
  - eAG (mg/dL) = 28.7 x HbA$_{1c}$ (NGSP %) – 46.7

eAG (mg/dL) = 28.7 x HbA₁C (NGSP %) – 46.7 \quad r = 0.92

Limitations of eAG

• Racial/ethnic limitations
  – Study was primarily conducted in Caucasian subjects
  – Different relationships between eAG and HbA$_{1c}$ in Caucasian and African subjects, not statistically significant
  – Certain ethnic groups were not included
    • Subcontinent of India (specimens delayed in shipment)
    • Pacific Islanders

• Some groups of subjects were not included
  – Children
  – Pregnant women
HbA\textsubscript{1c} and Race

- African Americans have higher HbA\textsubscript{1c} results than non-Hispanic whites
  - ~0.65% in diabetic patients
- African Americans have a higher HbA\textsubscript{1c} for a given glucose concentration that whites
  - Difference is 4% at HbA\textsubscript{1c} of 7% (~0.3% absolute)
- American Indians and blacks with IFG have higher HbA\textsubscript{1c} results than whites
  - Whites 5.78%, Hispanics 5.93%, Asians 6.00%, American Indians 6.12%, Blacks 6.18%
Hemoglobin Variants

- Common variants in some populations
  - African and Mediterranean populations
    - Hb S
    - Hb C
  - Asian populations
    - Hb E
    - Hb D
HbC & S Trait

HbC & S Trait

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* HbF levels above 15% cause clinically significant low bias
+ Offline manual recalculation must be performed if the HbF peak is mislabeled as labile HbA1c (LA1C)
Routine Laboratory Tests

- **HbA$_{1c}$**
  - 2 times per year if stable and meeting treatment goals
  - 4 times per year if therapy changed or not meeting treatment goals
- **Fasting lipids**
  - Annually, LDL-C target <100 mg/dL, <70 mg/dL optional
- **Liver function tests with further evaluation for fatty liver or hepatitis if abnormal**
- **Urine microalbumin**
  - Yearly, after 5 years in type 1 and in all type 2 patients
- **Serum creatinine and estimated GFR**
- **Urinalysis for ketones, protein, sediment**
- **TSH in all type 1 and if clinically indicated in type 2 patients**
Health Care Delivery Challenges

• HEDIS data for 2008 (median for commercial, Medicare, Medicaid)
  • Yearly HbA$_{1c}$ 77-88%
  • HbA$_{1c}$ >9% 29-48%
  • Yearly lipid profile 71-86%
  • LDL <100 mg/dL 31-47%
  • Yearly microalbumin 74-86%
Summary Points

• There are many different HbA$_{1c}$ methods
• Certification of HbA$_{1c}$ methodologies by the NGSP improves accuracy
• Laboratories need to be aware of potential interferences
• The greatest challenges are in appropriate test utilization and patient compliance
References

• DCCT Summary

• UKPDS Summary

• Lab Guidelines & Recommendations

• NGSP web site
  – http://web.missouri.edu/~diabetes/ngsp.html