Glycated hemoglobin (HbA1c)

Introduction

- Diabetes mellitus, as previously stated is a condition of hyperglycemia.
- It is estimated that this condition affects 2.5-5% of the population and is considered to be the fifth leading cause of death in the U.S.
- The disease is associated with a number of serious micro and macro-vascular complications involving the eyes, kidneys, heart and blood vessels, and may greatly impair the quality of life or shorten the life-span of the person afflicted.

Introduction

- There is a relationship between control of the glucose concentration fluctuation and the progresquantsion of the disease complications.
- There should be a method to quantify accurately and objectively the degree of altered blood glucose control over a long period of time.

Glycosylated hemoglobin

- In adults, hemoglobin is a mixture of three forms: Hb A1, Hb A2,Hb F, with Hb A1 predominating.
- Hemoglobin A1 consists of three subforms: Hb A1a, Hb A1b and Hb A1c, with Hb A1c predominating.
- The term glycated hemoglobin describes a chemically stable conjugate of any of the forms of hemoglobin with glucose.
- Glycated forms of hemoglobin are formed slowly, nonenzymatically and irreversibly at a rate that is proportional to the concentration of glucose in the blood.

Glycation: Nonenzymatic addition of a sugar residue to amino groups

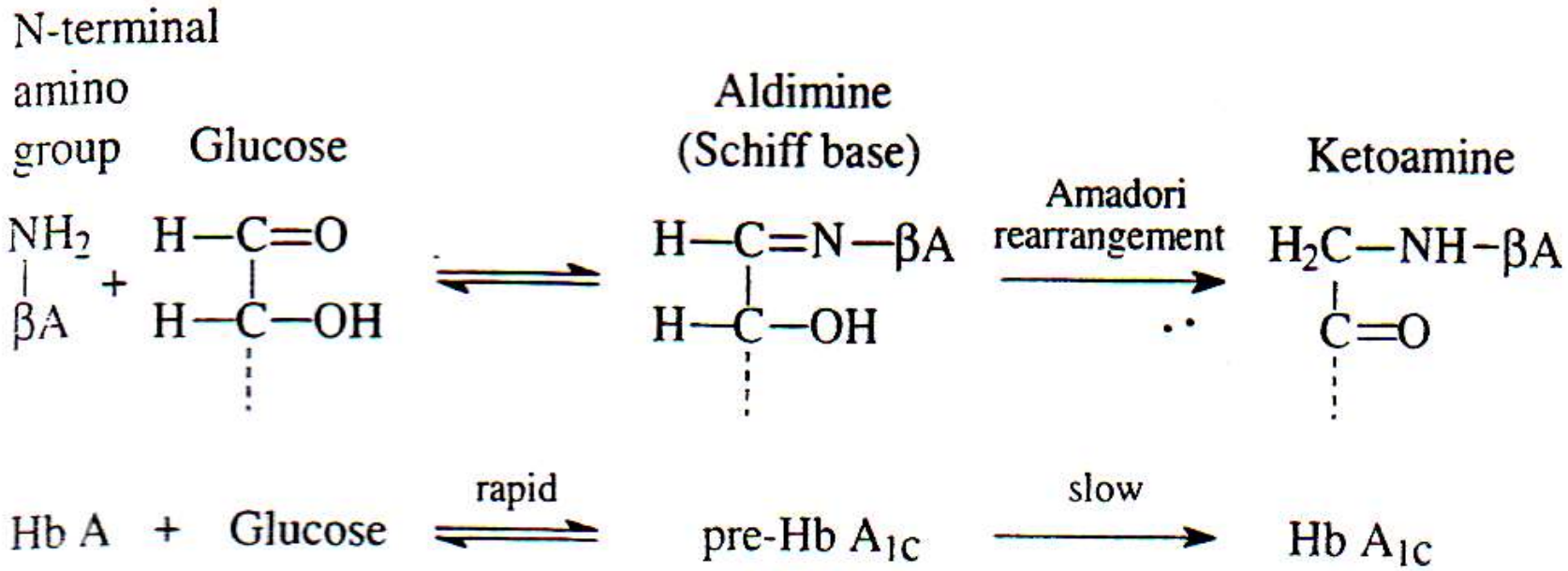

FIGURE **24-15.** Formation of hemoglobin A_{1c}.

Significance of test

- By testing for glycosylated hemoglobin, the doctor discovers what the average blood glucose level has been for the previous 2 to 3 months.
- This is especially valuable when monitoring diabetics whose blood sugars change dramatically from day-to-day and to monitor long-term diabetic control.
- Free of day to day fluctuations
- Unaffected by exercise or recent food ingestion

Glycosylated hemoglobin

- Blood levels of Glycated hemoglobin
  - Depends
    - on the life span of red cells
    - the blood glucose concentration

Specimen

- EDTA is the anticoagulant of choice for all methods.
- No special preparation, fasting specimens are not required.
- Most methods require cell lysis with a hemolyzing reagent provided by the manufacturer prior to loading.
- Typically, whole blood may be stored up to 7 days at 2-8^{0} C.
- heparinized samples
  - should be assayed within 2 days and may not be suitable for other methods (electrophoresis

Methods:

1. Cation-exchange chromatography
2. Affinity chromatography
3. Immunoassay.
4. Gel electrophoresis.
5. Enzymatic assay

Immunoassay

- The latex enhanced immunoassay for HbA1c is based on interactions between antigen molecules(HbA1c) and HbA1c specific antibodies coated on latex beads.
- This cross-link reaction results in changes in the solution turbidity which is proportional to the amount of the antigen in the samples .

Affinity chromatography

- Principle
  - m-aminophenyl boronic acid is immobilized by cross linking to beaded agarose or other matrix (e.g., glass fiber)
  - The boronic acid react with the cis-diol groups of glucose
  - Dissociation
    - By Sorbitol
  - Detection
    - Absorbance of bound and non bound fractions measured at 415 nm

Ion exchange chromatography

- Hemoglobin variants are separated based on charge difference
- Bed
  - cation exchange resin (negatively charged)

Practically:

- **The principle**
  - Cation exchange chromatography
- **Procedure**
- Preparation of hemolysate.
- Preparation of column:
  - Bring the column to room temperature
  - Remove the caps
  - snap the tip off the bottom.
  - push the upper disc down to the resin surface.
  - Let the column drain completely to waste.

Cont:

- Elution .
- Application of HbTOTAL .
- Detection: absorbance at 415nm.

- Calculation:

$$\%HbA1c = \frac{\text{HbA1c fraction}}{\text{Hb TOTAL}} \times \%100$$

Glucose mg/dl = (HbA1*35.5)-77.3

Reference range

- Glycated Hb is expressed as a percentage.

<6.4%	Normal(non diabetic)
6.5-8.4%	Good control
>8.5%	Not control

Interpretation of Glycated hemoglobin

- **Glycated Hb should be routinely monitored at least every 3 month in all insulin treated patients**
- **sources of errors**
  - **Low Glycated hemoglobin**
    - hemolytic disease
    - shortened red blood cell survival (sickle cell disease)
    - recent blood loss
  - **High Glycated hemoglobin**
    - Iron deficiency anemia
    - the effect of hemoglobin variants such as Hb F, S and C