Glycated Proteins

* Glycated Hb :-

→ nonenzymatic addition of sugar to amino group of protein

↓

k/a " glycation "

→ measurement monitoring of glycated Hb provides retrospective index of glucose value for long term glucose control.

Types of Glycated Hb

- HbA$_0$
  - ↓ Glycation ε lysine residue (n-terminal) &/or ε α-chain
- HbA-1
  - HbA$_{1a}$
    - HbA$_{1a_1}$ ↓ Fructose-1-6 Bis diphosphate attach to (valine) N-terminal β-chain.
    - HbA$_{1a_2}$ ↓ G-6-P attach to N-terminal valine of β-chain
  - HbA$_{1b}$ ↓ Pyruvic acid to N-termin valine of β-chain
  - HbA$_{1c}$ ↓ glucose to N-terminal valine of β-chain

* Total glycated Hb :— $HbA_{1c} + HbA_{1a} + HbA_{1b} + HbA_0$

→ Formation of ~~HbA~~ GHb is irreversible
↓
its Blood conc. depends on life span of RBC & Blood glucose level.
↓
it represents integrated value for glucose over previous 8 to 12 wks.
↓
① { Not affected By Day to day glucose Fluctuations, Recent exercise, food ingestion. }

* Interference in interpretation of ~~HbA1c~~ GHB :—

① pt c hemolytic d'se :
↓
Shortened RBC survival
↓
↓ GHB

② Recent significant Blood loss :
↓
high proportion of young erythrocyte
↓
false low GHB value.

③ Iron - defi. anaemia : (may be due to lead poisoning, ~~alcoholism~~)
↓

high proportion of old RBC

↓

high GHB conc.

④ Hb variants like HbF, HbS, Hb C :

↓

may interfere in interpretation.

⑤ Carbamylated Hb:

↓

attachment of Urea c Hb

↓

tnt in large amount in renal failure

c may occur in diabetic pt.

⑥ Labile Intermediates : Pre-HbA1C :

Schiff Base : —

↓

it may be included in measurement of HbA1C

↓

false high results.

↓

labile fraction changes rapidly c acute change in Blood glucose conc.

↓

So not an indicator of long term glycemic control

↓

In the -nce of glucose, pre-HbA1C.

reverts to glucose & HbA

→ Procedure to eliminates PreHbA1c :—

(a) Wash RBC & incubate in Saline
(b) Boronate affinity chromatography

↓

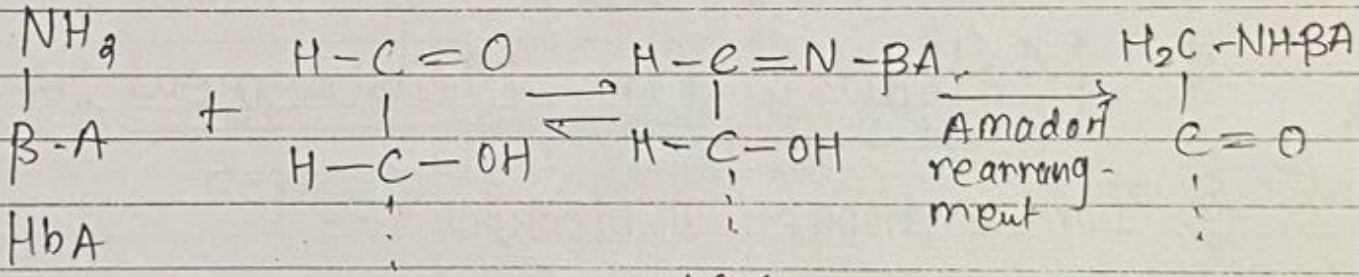
rapid dissociation of Schiff Base.

(7) drug like aspirin → causes formation of acetylated Hb.

✳ Formation of HbA1c :—

↓

N- terminal amino group — glucose



Schiff Base

HbA1c

HbA + glucose ⇌ (Rapid) Pre-HbA1c (slow) → HbA1c

✳ Clinical Utility :— Acc. to ADA.

HbA1c	DM
< 5.7 %	→ No Risk
5.7 – 6.4 %	→ high Risk of developing DM
≥ 6.5 %	→ DM.

→ HbA1c is useful for Dx's & monitoring of DM..
— identifying high risk pt
- for screening.

→ Result of HbA1c is expressed as a % of total Hb, any method used.

→ Selection of method depends on sample volume, pt popula^n & cost

→ ADA recommends that laboratory use only HbA1c assay that are certified By National Glycohemoglobin Standardiza^n programme (NGSP) & traceble to DCCT reference. (Diabete control & complica^n Trials)

* Methods for Measurement :—

① Ion exchange minicolumn :—

↓

Ion exchange chromatography, seperates Hb variants on the basis of charge

↓

Cation exchange resin, packed in disposable minicolumn

↓

affinity for HbA is tvely charged.

↓

pt Hemolysed sample is applied to the column

↓

Eluent collected. & contain

↓

Ionic strength & pH of eluent Buffers selected so that GHbs are less +vely charge than HbA

↓

So GHb elute 1st ($HbA_{1a} + HbA_{1b} + HbA_{1c}$)

↓

measured By spectrophotometer

↓

2nd Buffers of diff. Ionic strength added to elute main Hb.

↓

GHb is subjected to HbLC for sepeson of HbA1c from HbA_{1b} & HbA_{1a}

⟹ labile pre HbA_1 fracn, / elute ī / stable ketoamine

② HPLC :-

→ Hb A1C & other Hb fraction can be seperated by HPLC.

→ 5 ml of whole Blood

↓

in anticoagulant

↓

↓

anticoagulant Blood is diluted & Hemolysis
reagent containing Boronate → to remove glucose from labile fract^{n}

Can't remove glucose from HbA$_{1c}$ b'cos its irreversibly formed

↓

incubate 37°C for 30 minutes to remove schiff Base

↓

Now insested into auto samples

↓

a step gradient using 3 phosphate Buffers of ↑ing ionic strength is passed through column

↓

detec^{n} is performed at both 415 & 690 nm

↓

result is quantified by integrating area undes the peaks.

→ HbA$_{1c}$ By HPLC was used for analysis of all pt samples in DCCT.

③ Immunoassay :—

→ Ab^ against amadori products is formed or glucose
+
1st new amino acid at N- terminal end of β- chain of Hb

→ Principle : Inhibin of Latex agglutination

↓

Ab attached to latex Beads + Synthetic polymes containing multiple copies of immunoreactive HbA1C

↓

~~agglutinate~~ Add pt, sample Hemolyzed

Low conc. of pt HbA1C	High conc. of pt HbA1C
↓	↓
agglutination will occus	pt HbA1C compete ~~c~~ synthetic polymes to bind ~~c~~ Ab
B/w Ab + Synthetic polymes	↓
↓	~~No~~ inhibin of Agglutinan
more light scattering	↓
↓	↓ light scattering
↑ absorbance.	

→ Abs are specific for HbA1C

↓

So labile intermediates, Carbomylated Hb, & Othes variants not detected.

(2) Affinity chromatography :-

Immobilize Boronic acid

Resin + Glycated Hb $\xrightarrow{2H_2O}$ glycated Hb — Boronic acid — Resin

[comoplex]

↓

Non glycated Hb doesn't bind ☆ Boronic acid

↓

- elute 1st

↓

Sorbitol added to elute glycated Hb

↓

O.D at measure at 415 nm.

⇒ POCT method :- Affinity chromatography :-

→ Soluble reagent consisting of Dihydroxyboronate coupled ☆ HMW polyacrylic acid

↓

☆ Hb Binds to boronate

↓

This complex attaches to the cationic surface solid phase matrix

↓

Non glycated Hb remove in wash step as it is not bind

↓

Glycated Hb quantified By measuring quenching of fluorescence of fluorophore By Hb.

→ 2nd sample containing sorbitol

↓

will cause quenching of Fluorescence By removal of all Hb ⟹ quenching is done By Total Hb

↓

Sorbitol competes ⊂ ~~Ab~~ glycated Hb to binds ⊂ boronic acid

↓

Absorbance is measured ⊆ is of total Hb.

Adv :—

→ No interference from Non glycated Hb.
→ Unaffected By varia° in temp.
→ good preasion
→ Hb variant produce little effect
→ ↑

⇒ Detection of HbA1C from an equation obtained from linear regression B/w total glycated Hb & HbA1C analysis By HPLC

* Assay standardization :—

↓

→ NGSP established to implement the protocol to calibrated glycated Hb results to DCCT equivated values

↓

Network of Ref. Lab. by NGSP

↓

Interact ⊂ Menufactures of glycated Hb methods to help them calibrate their methods & values to DCCT.

Inc.

→ Certification is given only if manufactures performs precision testing.

→ This calibration efforts markedly improve harmonization result & reduce imprecision.

→ The ADA recommends that clinical lab. use only assays certified by NGSP.

* Reporting of HbA1C :—

→ (%) of Total Hb acc. to NGSP system
→ IFCC method : mmol/mol

→ Comparision B/w these 2 method

masters equation produced values helps to convert B/w this 2 reh. System.

eg: HbA1C result 7% is equal to 53 mmol/mol IFCC unit

→ Estimated Avg. glucose can be calculated from HbA1C measurement.

EAG (mg/dl) = 28.7 × HbA1C − 46.7

→ EAQ facilitates communicaⁿ c pt

* Specimen collection :-

↓

→ Nonfasting sample
→ EDTA, oxalate, floride vacutte

* ~~ADA~~ Acc. to ADA :-

↓

→ HbA1c should be routinely monitored atleast @very 6 months in pt who have stable glycemic contool. (for Both D.M I & DM II)

→ 1% change in HbA1c, 30 mg/dl change in @vg. glucose conc.