Controlled Molecular Engineering in T2DM (Possible future directions)

Sir, — With the given clinical protocol of repeat Blood Glucose estimations at regular intervals along with interpretation of repeat values of Hemoglobin Glycation (HBA1c) do give reasonable indications in clinical diagnostics. But definite *Prognostic and Predictive Diagnostics* however is still in early stages of development[1].

Question that remains:

HBA1c — Is it a comprehensive parameter for both Diagnosis as well as Prognosis (walk of the disease), or a half-done analyte we are happy with?

Looks like it's time this question should be raised for better following of the disease with one or more direct indicator(s) of ischemia being brought into consideration rather than simply following prevailing five types of blood glucose values at a given time as indirect presumptive indicators !

HBA1c, molecularly depicts level of non-enzymatic glycation of hemoglobin, a flowing phase protein, which gets anyway eliminated from system every 100 days +/-. This value is a glycemic indicator rather than depicting any irreversible Ischemic damage done by the C-6 carbohydrate.

T2DM damages are mostly concentrated in exchange bed capillaries in all organs where size of capillaries varies between 0.2 to 10 micro-meters. While entire human system has this exchange bed capillaries, maximal clinical expressions come from kidney,eye,brain,heart, skin and peripheral vessels.

Molecular basis of these damages, collectively called — Vasculitis — are as follows:

Glucose exists in two forms :

@ alpha-d-glucose

@ beta-d-glucose

And as their racemic mixture.

Both alpha- and beta-forms are closed ring forms with a few exposed least reactive hydroxyl groups (Fig 1). Oral carbohydrate consumption leads to a buildup of blood level of these two forms, finally settling to a mixed form mentioned.This interchange takes place within aqueous phase of blood where alpha-d-form muta-rotates to the beta-d-form and vice-versa finally settling to a racemic mix of the two. The process, as mentioned, is known as mutarotation.

What is extremely important is the fact that the bidirectional transformation passes through an open-chain form of glucose molecule for an extremely tiny fraction of a second with a hyper-reactive aldehyde group getting exposed (possibly this transition takes place in a time frame of pico-second or even less).

This super-fast hydrated-aldehyde is the central offending group of Glucose which reacts with nearly anything and everything in both *flowing phase* and *fixed phase* of blood, of which our clinical measurements, till date, have remained confined to Hemoglobin Glycation only, a flowing phase phenomenon !

Given this molecular transformation as background, important question that arises is as follows :

Do we need a new *prognostic parameter*?

Perhaps yes.

HBA1c is a globally accepted diagnostic indicator, but it hardly tells anything about the molecular pathology of vascular occlusion and resulting immunological reactions — collectively known clinically as Vasculitis.

Essentially Stage - I damage in T2DM is nothing but caused by the open-frame glucose molecule in muta-rotation with generated hyper-transient-hyper-reactive -CHO group getting adhered to the fixed phase exposed reactive groups (-NH_2, -COOH, -OH and -C=O) on the surface of endothelial cells in micro-capillaries across the organs, in addition to forming AGEs.

Deficiency in our ways of looking at T2DM lies in:

@ following the offending agent,glucose, in various forms only,

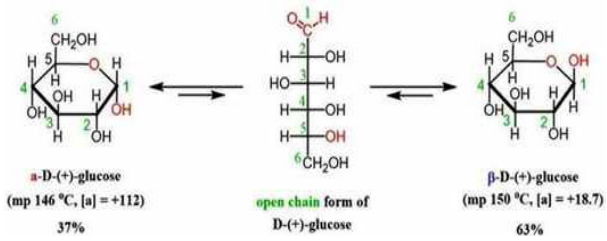

Fig 1 — Schematic presentation of mutarotation, a textbook material

@ our efforts of having peripheral and indirect assessment of progress of the disease (prognosis) from glycation of a flowing phase protein, Hb, whereas till date there is no stable reproducible parameter of individual organ and or system ischemia which is the core of Diabetic molecular pathology.

To have a more comprehensive assessment of disease progress, we should have more direct assessment parameter(s)–like *differential organ specific and collective ischemia indicators* caused till date by the pico-second-nonspecific-nonenzymatic-glycation mediated by the hyper-transient aldehyde group (of 10^{-12} to 10^{-11}sec lifespan).

Needless to mention here that glycation is an irreversible process, essentially meaning — once glycosylated the adherent glucose cannot be removed. Hence, our knowledge of *Fixed Phase Glycation* (wall glycation) is of utmost importance given that the glycated wall proteins cannot be cleaned or the accumulated carbohydrate loci on micro-vascular wall cannot be removed physically in micro-exchange-beds and filter-beds, however reduction in glucose load might be achieved through Continuous Blood Glucose (CBG) monitoring and subsequent intense therapy ! And these wall accumulations in bends and corners of micro-vascular beds become secondary adherence concentrators and attachment points for activated platelets, other glycated proteins, cells with wall glycation loads, all forms of lipids and lipid-protein complexes finally resulting in fixed occlusive and flow restricting areas (points of augmented atheromatous plaque formation).

Hypertension adds to the wall injury because of high shear on capillary endothelium resulting in pre-formation of wall attachment sites for activated glucose (with active aldehyde) and other molecular invitees .

Possible Directions

(1) Inhibition or time-bound suppression of free aldehyde formation through time-controlled systemic alkalinization (pH 7.35 raised to pH 8.0).

Work is in progress for optimization of :

a) use of commercially available alkali water,

b) estimating duration of elevated pH holding vis-à-vis blood glucose levels,

c) initiation of any other adverse effect(s) in patient groups with and/or without oral hypoglycemic/insulin therapy in fresh (<2 years) and standing diabetic cases (>2 years) under active therapy and without diabetic complications (unpublished work).

While attention got focused on degree of angular shift of polarized light, the point of equilibrium, equilibrium composition and details of bond chemistry the biological importance of the hyper-transient intermediate straight molecule got to the backbench of consideration. While mutarotation is a physical phenomenon of any optically active molecule, it acquires a profound significance in case of C-6 hexose Glucose because of the in-between hyper-reactive hyper-transient aldehyde (of 10^{-12} sec +/- duration). While alpha-D and beta-D forms are nearly non-reactive from glycation point, generated aldehyde (-CHO) is a highly reactive group reacting with all other reactive groups mentioned above.

While clinical evaluation of glycation is done only in mobile-phase intra-RBC protein Hemoglobin, ideal assessment of glycation should be in both @ flow-phase and @ fixed phase proteins to

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have a comprehensive assessment of both @ glycation induced incapacitation of intra-cellular, cells-surface and free flowing proteins and @ degree of ischemia building up because of irreversibility of wall-glycation process.

Alkaline pH profoundly reduces half-life of aldehyde group because in alkaline pH aldehyde reacts with hydroxide ion leading to formation of stable end products (germinal diol or enolate ion). Alkaline pH also stabilizes glucose in its cyclic form thereby inhibiting aldehyde generation by slowing down mutarotation. *Pre and Post-meal-controlled-alkalinization* through optimized volume of alkali water possibly buys time for blood level of free glucose to get transported to intra-cellular compartment. While all other therapeutic approaches and molecules remain in play this new molecular engineering adds up in reducing extra-cellular glucose derived aldehyde and its free play in irreversible glycation of micro-capillary fixed phase proteins.

A couple of other small molecular agents – Carnosine and Lysine – to mention a couple are under active studies and screening (unpublished data)

(2) Use of conventional therapy: All forms of conventional therapeutic modes – Small molecules, Protein, Peptide(s), Fibers, Residual and Controlled Carbohydrate Diets will add-up significant value to this molecular modification approach which basically intends to reduce –CHO group generation, wall adherence of glucose and building up of *irreversible ischemia* resulting from *flow-hindrance and diffusion hindrance*.

(3) To look for reproducible robust indicator(s)of *accumulating ischemia* for objective in-therapy prognostic evaluation. Built-up-irreversible-ischemia at a given point remains completely out-of-sight of clinical considerations –now— despite very controlled glucose load achieved through rigorous therapeutic regimes. Ideal therapeutic screen at any given point must have close control of @ dynamic glucose load and @ ischemic load at that point.

(4) Induction of controlled angiogenic response – A possible new complimentary mode of therapy : Glycation being irreversible, mobile phase glycation and fixed phase glycation have different endpoints. While mobile phase glycation – HBA1c, AGEs and glycated cells are removed from circulation continuously as a function of time , fixed phase glycations – mostly endothelial surface glycation in exchange and filter beds need special attention :

@ to be either cleaned or, @ to be replaced (In-situ repair).

While in-situ cleaning is not in focus globally, in-situ replacement of capillary exchange beds, with prospect of controlled induction of controlled angiogenic response in ischemic tissues possibly constitute the final frontier[2-9]. Encouraging results obtained thus far may hold a true promise in in-situ repair of ischemic tissues and organs through a set of Low-Mol-Wt angiogen(s) with a new therapeutic direction – Controlled Reperfusionof Ischemic Tissues Through Low-Mol-Wt-Angiogenic(s), both being globally new directions. Further molecular and clinical consolidations are obvious next steps.

Conclusion

(1) T2DM now is possibly looked at partially through estimations of offending agent in different formats.

(2) Best glycation parameter depended on now, HbA1c, indicates degree of flowing-phase-glycation (glycemic index and is not an ischemic indicator).

(3) No fixed-phase-glycation data is available now.

(4) Irreversible accumulating exchange-and-filter-bed ischemia from fixed-phase-glycation constitutes the core of molecular pathology, which is not looked at by a long margin. Reduction and Control of blood glucose to a permissible level donot address past glycations resulting in summated ischemia – both systemic and organ specific. Not all diabetics are equal and uniform glycators. Different wall glycation loads and different anatomical locations at advancing stages of T2DM act as variable degrees of *Ischemia Concentrators* and *Flow Hindrancers* (Wall glycation coupled with Flow glycation – in combination – induce both flow-hindrance as well as diffusion-hindrance).

(5) Preferential organ damage, if any, like kidney versus eye, should have definite indicator(s), marker(s) — analyte or genetic .

(6) T2DM is an ischemic disease and must have ischemia diagnostics and Ischemia therapeutics.

(7) Predictive diagnostics – overall – are at preliminary stages although organ specific prediction possibility holds good prospect.

(8) Controlled Reperfusion of Ischemic Organs and Tissues particularly of T2DM origin is a clear possibility with initial technology-demonstration-studies have already proven promising – in in-vitro cell culture, in small animals and in human study. More organized broad based studies needed.

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