Haemoglobin A_{1c} and Diabetes Mellitus
(A brief review)

1. Structure

In an adult, major form (90 percent) of Hb is Hb A with a subunit structure of $\alpha 2\ \beta 2$, The remainder consists of Hb A_{1a} (1-2 percent), Hb A_{lb} (1-2 percent) and Hb A_{1c} (4-6 percent) and possible other minor components Hb A_{ld} and Hb A_{le}.

The minor haemoglobins can be separated from Hb A by various chromatographic procedures, at a slightly acidic pH 6.7, the minor haemoglobin being more negatively charged than Hb A. In Hb A_{1c}, there is a negatively charged group (glucose in ketoamine linkage) to the N-terminal end of the Beta chain and it has been shown that Hb A_{lc} is the result of glycosylation of Hb A, this reaction occurring in the red blood cells in the peripheral circulation. The glycosylation reaction is non-enzymatic while its rate depends on the blood glucose level and the duration of the red cells exposure to blood glucose. Thus concentration of Hb A_1C at any given time reflects a patient's mean blood glucose level for preceding weeks to months-time averaged concentration of glucose within the erythrocyte.

There is an unstable component of Hb A_l which perhaps represent the Schiff base and probably is formed as the initial step in formation of glycosylated haemoglobin. Short term hyperglycaemia can modify Hb A_1 unstable content by about 3% or more.

It is likely that synthesis of Hb A_{lc} may represent a model reaction to explain the biochemical basis for many of the long term sequlae of diabetes. The tissues that suffer the most noticeable dysfunction is diabetes (e.g. retina, lens, peripheral nerve, kidney) appear to be insulin independent for glucose uptake. Perhaps in diabetes the intra-cellular proteins of these tissues undergo excess non-enzymatic glycosylation, analogous to that seen within red cells. Such glycosylation might alter the enzymatic activity, solubility, antigenicity and other function of the protein and thereby result in the observed clinical dysfunction. Again, Hb-A_{lc} has decreased reactivity with 2, 3 DPG so red cells of diabetics will have increased affinity for oxygen, this results in shift in the oxygen dissociation curve and tissue hypoxia. If this becomes chronic, it could be contributory to long term microvascular complications.

2. Methods of estimation :

These include Ion exchange chromatography, Gel electro-focussing, Radioimmunoassay, and Colorimetric tests. In most laboratories, method of chromatography on cation exchange columns is followed. Detailed description is as reported by Gabby et al (1977).

Various causes of variations in estimation should be familiar and these are : (a) Conditions and time of storage of samples before analysis, (b) Method employed, especially the temperature of chromatographic column, and (c) the content of unstable component (upto 3%) that gets incorporated.

Normal values are between 4-8% of Hb A. *Low values* are present in haemolytic anaemia (shortened span of RBC), haemoglobinopathies and in instances undergoing venesections for haemochromatosis (faster turnover of RBC). Lower than expected values are reported in pregnant diabetic due to influx of young red cells during pregnancy.

High values are present in iron deficiency anaemia, cystic fibrosis.

Both low and high values are reported in instance with uraemia. Alterations with age are also reported.

3. Diabetes and Hb A_{1c}

Fasting blood glucose, mean daily plasma glucose value (frequent glucose determination over a 24 hour period) and highest plasma glucose concentration attained during 12 hour of continuous glucose monitoring bear significant correlation to Hb A_{lc}. Hb A_{lc} level reflects the integrated sum of glucose levels over a period of a few weeks, and thus provides clinical application in the assessment of the long term blood glucose cantrol. Some other states in diabetes, where Hb A_{1c} values are altered.

(a) In diabetic ketoacidosis values are high.

(b) In context of lipid profile, there is significant correlation between fasting cholesterol and trigly-cerides, though values for correlations are low.

(c) Diabetes pregnancy-In gestational diabetes values are significantly raised. Also maternal Hb A_{lc} relates to the birth weight. In pregnant diabetics regression line shows a close correlation between fasting blood glucose and Hb A_{lc}.

Following limitation regarding Hb A_{lc} value in diabetes need recognization :

(a) In those genetically predisposed (both parents diabetic or an identical diabetic twin), Hb A_1 values are normal. In IGT as well, Hb A_{lc} is not a sensitive indicator.

(b) As well in those with complications (vascular),no correlation with Hb A_{lc} measurement has been authenticated.

4. Practical Advantages

4. The practical usefulness of Hb A_{lc} determination can thus be summarized as follows :

(a) Single value indicates the metabolic control.

(b) It is independent of time of day, meals, exercise.

(c) Valuable in patients in whom-renal threshold for glucose is abnormal or patients with unstable insulin dependent diabetes.

(d) In follow up Hb A_{lc} value simplifies interpretation, series of values over months/years is very useful in profile assessment.

(e) In deciding if treatment modality needs a change, e.g. oral to insulin, as well represent an accurate technique to evaluate new way of controlling blood glucose.

Some of the *disadvantages* are as follows :

(a) It does not permit acute modification in insulin or oral therapy.

(b) Hb A_{lc} does not reflect occurrence of hypoglycaemic episodes.

(c) Severely or poorly controlled patients can have normal Hb A_{lc} values.

5. Questions that seem unresolved so far can be stated as follows :

(a) What is the minimal blood glucose and time product that will result in a demonstrable increase in Hb A_{1c} concentrations ?

Significant variation over 24 hours (though diabetes have higher baseline values and less variation) are reported. In diabetics 4 days of poor control induced significant rise of Hb A_{lc} levels. Short term elevation raises the unstable form of the Hb A_{lc}.

(b) Is glycosylation of structural protein, such as those in basement membranes in various organs related to Hb A_{1c} ?

It is known that changes in other glycoproteins are occurring in diabetes, thickened basement membrane of diabetic glomeruli is composed of abdormal glycoproteins selectiveiy enriched with carbohydrates. Again, quantitative changes in fibrinogen and Von Willebrand factor related to glycoproteins also occur in diabetes. In cataract also, lens protein undergo non-enzymatic glycosylation of lysine amino groups in the presence of high glucose environement, high molecular weight aggregate thus formed diffract right and result in opacities.

(c) What is the extent of reversibility of formation of Hb A_{1c} ?

Short lived changes in blood glucose in normal case also affect the Hb A_{lc} content. The Hb A_{lc}, however, does not reflect blood variation within the normal range nor assist to detect IGT instances.

Selected References

1. Editorial-Hb A1 and diabetes, a reappraisal. Brit. Med. J. 281, 1304, 1980.

2. Gabbay, K.H., Hasty, K., Breslow, J.L., Ellison, R.C., Bunn, H.F., Gallop, P.M. Glycosylated haemoglobin and long term blood glucose control in diabetes mellitus J. Clin. Endocrinol. Met. 44, 859, 1977.

3. Gonen, B., Rubenstein, A.H. Haemoglobin A_1 and diabetes mellitus. Diabetologia, 15, 1, 1978.

4. Gonen, B., Rubenstein, A.H. Glycosylated haemoglobins in diabetes-a reappraisal. Diabetes Care, 2, 451, 1979.

5. Rochman, H. Haemoglobin A_{lc} and diabetes mellitus. Ann. Clin. Ft Lab. Sc. 10, 111, 1980.