

## ABSTRACT

The relative importance of the measurement of glycosylated hemoglobin as an objective and quantitative index reflecting blood glucose levels of diabetics during the preceeding four to six weeks is now well established. Similarly glycosylated plasma proteins could be used as an index of short term glycaemic control.

No previous investigations have been carried out in Sri Lanka on glycosylated hemoglobin and plasma proteins. Therefore a suitable technique has been evaluated for the assay and the clinical norms established.

The spectrophotometric method originally described by Flückiger and Winterhalter (1976) and subsequently modified by Worth et al (1978) was evaluated and adopted as a suitable technique for the assay.

A linear increase in colour development was observed upto 75 g/L total hemoglobin concentration.

Incubation with oxalic acid for one hour indicated good precision with coefficient of variation within assay of 3.19 (n=20) and between assay of 2.8% (n=15) for normals and 1.8% for diabetics. Hemoglobin standards prepared were stable for more than six months.  $\beta$ -D-fructose was also used in addition to 5-hydroxy methyl furfuraldehyde as standards. This gives a measure of efficiency of the reaction. The non specific blank value for all samples were a constant. Similar findings were observed with glycosylated plasma proteins.

Whole blood samples could be stored at room temperature ( $30 \pm 3^{\circ}\text{C}$ ) for 5 days and for nine days at  $4^{\circ}\text{C}$ . Hemolysates were stable at  $4^{\circ}\text{C}$  for 5 days and for 30 days at  $-20^{\circ}\text{C}$ .

A good correlation coefficient of  $r=0.92$  was obtained with a microcolumn test kit (n=20,  $p < 0.001$ ).

The normal mean for glycosylated hemoglobin ( $\text{HbA}_1$ ) for Sri Lanka was found to be  $5.85\% \pm 0.79$  for males (n=70) and  $5.88\% \pm 0.79\%$  for females (n=30). A significant difference was not observed between males and females. The overall

mean for normals was  $5.9\% \pm 0.79\%$  (or  $0.39 \pm 0.05$   $\mu\text{M}$  HMF/g Hb). Mean for non insulin dependent diabetics was  $10.83\% \pm 3\%$  (or  $0.68 \pm 0.20$   $\mu\text{M}$  HMF/g Hb). The mean %HbA<sub>1</sub> for pregnant women (n=15) in the 3rd trimester of pregnancy was  $7.0\% \pm .7$ .

The results are in agreement with those reported earlier.

The normal mean (n=20) for glycosylated plasma proteins was found to be  $0.25 \pm 0.092$  n HMF/mg protein and for non insulin dependent diabetics  $0.42 \pm 0.11$  n HMF/mg protein.

Good correlation coefficients were observed between %HbA<sub>1</sub> values and both glycosylated plasma proteins and fasting blood glucose.

Average time of assay for 30 samples was  $3\frac{1}{2}$  hrs. The cost per assay is low and could be performed weekly after the collection of a number of samples.

The colorimetric method meets many of the criteria for an ideal laboratory test. It proves to be the most suitable method for the measurement

of glycosylated hemoglobin and plasma proteins  
as a routine assay in general and regional  
hospitals in Sri Lanka.