

Symposium :

GLYCOSYLATED HEMOGLOBIN

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Allen in 1958 showed chromatographic heterogeneity of hemoglobin A (1). Rahbar in Taheran first demonstrated elevation of minor hemoglobin in diabetes mellitus in 1968 (2). Trivelli introduced the column method of separating out the fast hemoglobins in 1971 (3). Bunn and co-workers in 1978 pointed out that hemoglobin A_{1c} is related to control of diabetes (4). The column method used in other countries, both the micro and macro columns have been expensive and as we shall discuss later, they are subject to more errors than the chemical method. Therefore, we have been interested in the chemical method that was described by Fluckiger and Winterhalter in 1976 (5). We modified this method to some extent to suit our needs and have tested it out over the past 5 years and found it to be working satisfactorily (6). This method has the advantage of not being influenced by changes in the ionic charge of the hemoglobin which occurs in many hemoglobinopathies and which occurs following aspirin or alcohol ingestion. The only significant factor which alters the result is the altered life span of R.B.C. The method is exacting, but is feasible in any standard laboratory.

With the extensive studies that Bunn and co-workers carried out and published in 1978, glycosylated hemoglobin is now established as an excellent method to monitor the control of diabetes (4). The chemical method that we employ estimates total glycosylated hemoglobins of which the major component is hemoglobin A_{1c}. However, it also includes hemoglobin A_{1b} and hemoglobin A_{1a}. Glycosylation reaction occurs with the terminal valine of the amino group of hemoglobin molecule. As a result initially an unstable compound called aldamine is formed. This compound is probably same as what we now label preglycohemoglobin. With Amadori rearrangement, this aldamine changes to a stable ketomine compound. In the chemical method that we employ this ketomine is reacted with thiobarbituric acid to generate furfural compounds which are estimated colorimetrically.

We followed three groups of diabetics prospectively over three-month period with repeated fasting and post-prandial blood glucose estimations and estimations of glycosylated hemoglobin at the end of the study period. We demonstrated that glycosylated hemoglobin method set up by us separated very clearly patients with good, fair and poor control of diabetes (Table 1). We also found a close correlation of blood glucose with glycosylated hemoglobin ($r=0.82$, $p<0.001$) (6).

Subsequent to this study we have applied this test routinely for the follow-up of our diabetics and about two years ago we reported results of 4203 estimations done in an out-patient clinic situation (Table 1). In this study, a spot blood glucose and glycosylated hemoglobin estimation was made. We wanted to see in what proportion of our patients glycosylated hemoglobin gives different information as compared to blood glucose estimation regarding control of diabetes.

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Table 1

Group	Control of diabetes, present and preceding 2 months	Fasting Blood Glucose \pm SEM mg/dl	2-hour post-lunch Blood Glucose \pm SEM mg/dl	Glycosylated Hemoglobin % \pm SEM
I	Good Control n=9	103 \pm 4	122 \pm 6	5.7 \pm 0.36
II	Fair Control n=13	133 \pm 7	155 \pm 15	7.7 \pm 0.31
III	Poor Control n=22	202 \pm 2.2	263 \pm 16.6	9.5 \pm 0.35

We termed results as "concordant" when glycosylated hemoglobin as well as fasting and post-prandial blood glucose estimation gave us the same interpretation regarding the control of diabetes, that is, both the tests showed that the control was good or fair or poor. We termed the results "partially different interpretation" when there was a one step difference, for example if glycosylated hemoglobin revealed that the diabetes was controlled poorly and blood glucose estimation revealed that diabetes was controlled fairly or vice-versa. We interpreted results as being "totally different" when glycosylated hemoglobin and blood glucose revealed entirely different results like one test revealed that diabetes was under good control while other test revealed that diabetes was poorly controlled. In this study we observed that about 50% of the patients had concordant interpretation while the remainder had different interpretation (of these about 4% had totally different interpretation while 46% had partially different interpretation) This study revealed that use of glycosylated hemoglobin is important clinically as in about half of the patient it gives you a different interpretation regarding metabolic control of diabetes than estimation of blood glucose alone. Usually, patient revealing poor or fair control of diabetes on the basis of glycosylated hemoglobin would reveal better control of diabetes by the blood glucose values. This shows that patients tend to diet better on the day of blood glucose testing, although we also found that a small number of patients were behaving vice versa.

We have applied glycosylated hemoglobin to all variety of clinical situations in diabetes. This is an excellent parameter to judge control of diabetes during pregnancy. It has been shown that the fetal malformation in diabetic pregnancy are related to poor control of diabetes during the early stage of embryogenesis. Diabetic fetopathy has become very rare at present, both in gestational diabetics and insulin-dependent diabetics with pregnancy. This is because of intensive insulin therapy undertaken in these diabetics. However, residual mortality in diabetic pregnancy is due to fetal malformation. This has been shown in experimental animals to occur during the first ten to twelve weeks of pregnancy. If you see a pregnant diabetic who has just missed her periods or who is two to three months pregnant, estimation of a single glycosylated hemoglobin at that time will give you information about the past

three months which will include time of conception. In case this value is normal, it is very encouraging, as the chances of fetal malformation would be smaller in that pregnancy. It is also important to follow glycosylated hemoglobin throughout the pregnancy. We have done it in all our gestational diabetics as well as insulin-dependent diabetics who became pregnant and we have been able to normalise this parameter in all our pregnant diabetics. In those pregnant diabetics, where glycosylated hemoglobin is normal to begin with and stays normal throughout the pregnancy, we often advise Obstretican to carry the pregnancy to full term instead of the usual advise of termination at 38th week.

Glycosylated hemoglobin has also been used to differentiate between stress hyperglycemia and diabetes. An elevated blood glucose value in a patient who has just been hospitalised with a pain of myocardial infarction or with a stroke or with an accident may not clearly tell you whether it is stress hyperglycemia or the patient has already been a diabetic for some time. In a study of 29 patients we have been able to show that an elevated glycosylated hemoglobin in this situation means that the patient is an established diabetic while a normal glycosylated hemoglobin means that patient has stress hyperglycemia. We have also observed elevation of glycosylated hemoglobin after 6-8 weeks of stress periods, paralleling the elevated blood glucose values during this period. We have also realised that stress hyperglycemia is not as transient an event as is usually believed and it leads to significantly increased glycosylation. These data have led us to believe that stress hyperglycemia should be treated (Fig. I) (7).

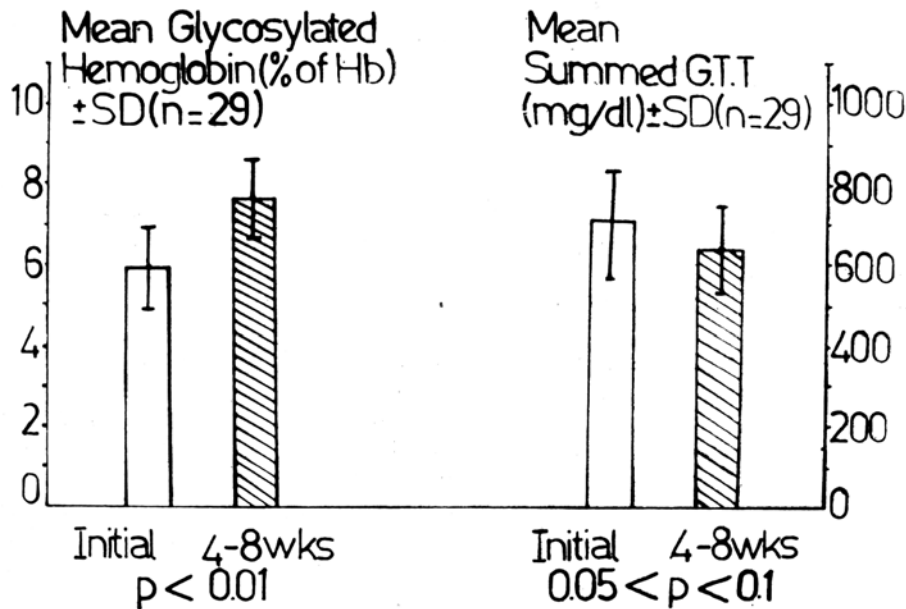


Fig. 1 : Glycosylated Hemoglobin and summed GTT initially and 4-8 weeks after vascular episode.

Table 2

Interpretation of Metabolic Control

Glycosylated Hemoglobin (GHb) versus Blood glucose (BG) (n = 4203)

	Percent
Concordant interpretation (n = 2097)	49.9
Different interpretation (n = 2106)	50.1
Partial (n = 1942) (46%)	
Total (n = 164) (4 %)	
GHb showing poorer control than BG (n = 1327)	31.5
GHb showing better control than BG (n = 779)	18.5

We have uniformly applied this parameter in diabetics who are planning to undergo surgery. With the stress and because of anxiety and immobilisation, the blood glucose values often rise in the preoperative period. However, if glycosylated hemoglobin is normal and the prevailing hyperglycemia is not marked we usually allow the patient to undergo surgery and we have had no complications by following this approach.

Glycosylated hemoglobin is now accepted to have several advantages over the conventional methods of estimating blood glucose to check metabolic control of diabetes. It obviously gives time-averaged blood glucose values over the past two or three months and is of great value in insulin-dependent diabetics. As is pointed out by Molnar, the insulin-dependent diabetics and specially a few so called "brittle diabetics" have such wide swings of hyper and hypoglycemia throughout the 24 hour period that whatever parameters you employ, for example, mean blood glucose or the mean amplitude of glycemia excursion or mean of daily differences, you do not get the exact idea of the metabolic control. In insulin-dependent diabetics, therefore, glycosylated hemoglobin forms a very important parameter.

In all research studies in field of diabetes, glycosylated hemoglobin will form a very important parameter. The long term studies regarding vascular complications of diabetes have been fraught with severe difficulties because of non-availability of this type of parameter in the past. We, have employed this method in several research problems, for example, we wanted to find out whether the metabolic control of diabetes deteriorates during the Ramzan fasting in our muslim population. As it is not possible to do repeated blood glucose sampling during the Ramzan period, we used this particular parameter to provide us the answer. Glycosylated Hb in the prefasting and fasting period was 8.14 and 7.52 per cent respectively and did not differ significantly. This study, therefore, led us to conclude that it is possible to undertake Ramzan fasting without any significant deterioration of metabolic control of diabetes (Fig 2).

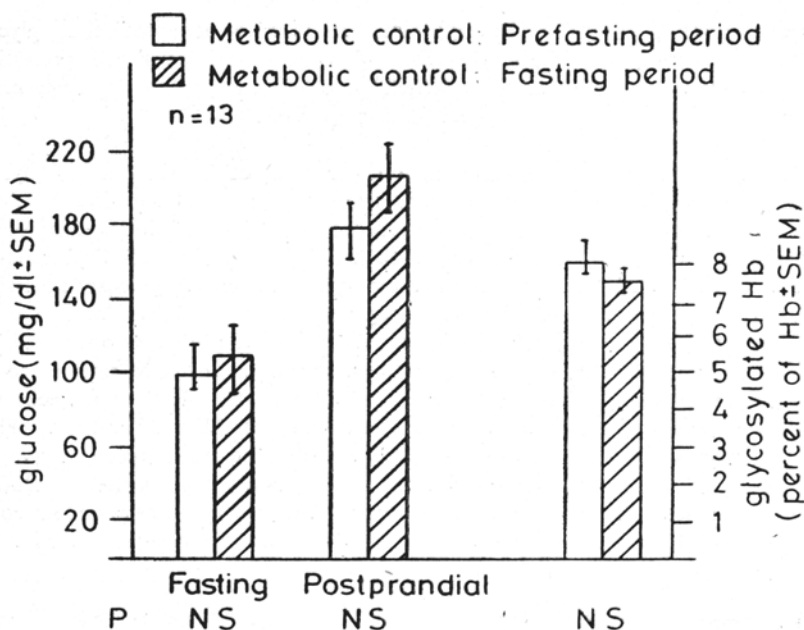


Fig. 2: Fasting, post-prandial Blood Glucose and Glycosylated Hemoglobin in the pre-fasting and fasting period.

A variety of factors can alter results of glycosylated hemoglobin estimation specially by the column method. The column method which is based upon the ionic charge of the glycosylated hemoglobin will show wrong result in the presence of a hemoglobinopathy. It is known that false low values are produced by hemoglobin S, C or D and spurious elevation occurs in the presence of elevated fetal hemoglobin. Other drugs which alter ionic charge of hemoglobin, like alcohol and aspirin can also alter the results with the column method. Column method requires exacting pH and temperature conditions. The chemical method used by us obviates all the difficulties and the only significant factor that alters the results is altered R.B.C. life span. It is interesting to note that in their original paper Bunn and his coworkers had also showed somewhat lower values of glycosylated hemoglobin in patients of hemolytic anemias. However, no systematic study in this direction has yet been reported. We have attempted to correlate glycosylated hemoglobin in patients of hemolytic anemia to their R.B.C. life span. We estimated R.B.C. life span by chromium tagged R.B.C. half life. The R.B.C. life span and glycosylated hemoglobin were significantly reduced in patients of hemolytic anemias ($p < 0.001$). It may be possible to judge the degree of hemolysis by estimating glycosylated hemoglobin.

A variety of questions regarding glycosylated hemoglobin have never been answered. It is well accepted that glycosylation is a process of degree of hyperglycemia over a certain period of time. However, no one has yet described clearly as to what degree of hyperglycemia is required for what length of time to cause an appreciable and detectable rise of glycosylated hemoglobin.. It is also

accepted that glycosylated hemoglobin is an estimation of average blood glucose values over the preceding three months. However, it is not clear as to which part of the period is most crucial or contributes maximally towards the glycosylation process.

We have conducted some studies on the rate of dissipation of glycosylated hemoglobin in a group of 13 newly detected, untreated, type II non-insulin dependent diabetics with markedly elevated blood glucose and glycosylation hemoglobin. We have controlled these patients vigorously with the administration of Glybenclamide or Glipizide and estimated glycosylated hemoglobin and blood glucose at two week intervals. Our study revealed that the drop of glycosylated hemoglobin is pari passu with the drop of blood glucose value and is most rapid in the initial two weeks. The rate of drop gradually declines and the glycosylated hemoglobin usually returns to normal in about 8 weeks time. Maximum drop is 1% over the first two weeks which is followed by diminishing drop every two week interval (Fig 3 and 4). This may indicate that glycosylated hemoglobin probably mirrors the more recent hyperglycemia events more closely than the ones in the past. There is a study reported by Boden and his coworkers which has also revealed that when therapy is withdrawn in controlled diabetics and blood glucoses are allowed to rise, maximum glycosylation occurs in the first two weeks (8).

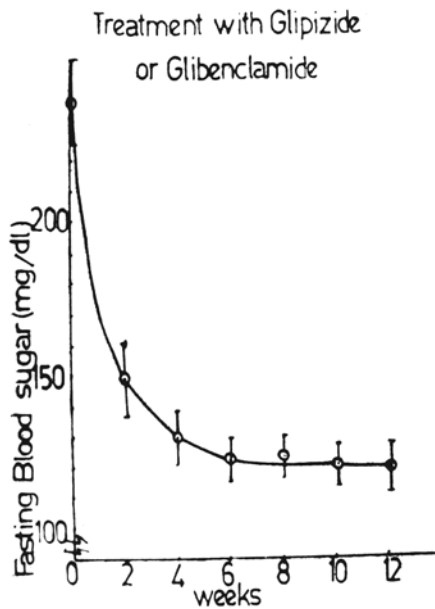


Fig. 3 : Fasting Blood Glucose at two-week interval with sulfonylurea therapy.

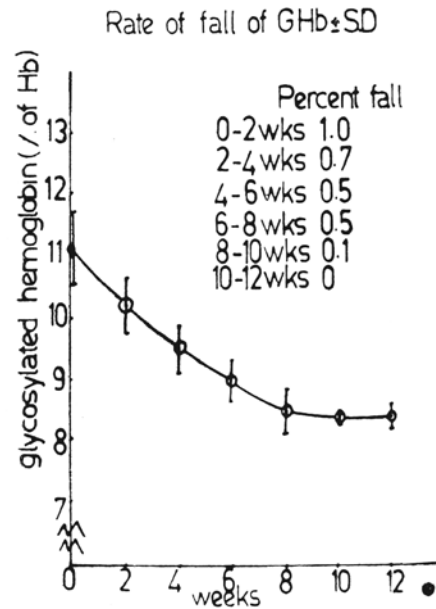


Fig. 4 : Glycosylated Hemoglobin at two-week interval with sulfonylurea therapy.

A few disadvantages of using glycosylated hemoglobin need to be pointed out. It is not possible to make alternations in the drug or insulin dosage on the basis of glycosylated hemoglobin. This has to be done on the basis of blood glucose. The cost

of glycosylated hemoglobin estimation is high, but is not more than 2-3 blood glucose estimations in our laboratory. The presence of pre-glycosylated hemoglobin may falsify the results in various glycosylated hemoglobin estimation methods. Pre-glycosylated hemoglobin has same electrophoretic mobility and it coelutes with the glycosylated hemoglobin on the columns that are used for the estimation of glycosylated hemoglobin. For this reason, various methods have been described, including a simple method of saline incubation of the R.B.C. for a period of period of about 6 hours to remove preglycosylated hemoglobin (9).

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