Original Research Article

Study of significance of glycosylated hemoglobin in diabetic patient

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Abstract

Background: Investigation of the structure and biosynthesis of glycosylated Hemoglobin (HbA1c) in the past decade have provided a means to objectively access the average level of glycemia in diabetic patient. The use of Glycosylated hemoglobin level as integrated index of long term blood glucose level, represent a significant tool in our research and therapeutic armamentarium. In this study, we have estimated glycosylated Hemoglobin (HbA1c) in diabetic and non diabetic person and its relationship with fasting and post prandial blood sugar levels.

Materials and methods: In present study, Glycosylated hemoglobin levels were estimated by using cation exchange resin method. The study was conducted from November 2012 to October 2014. Measurement of total HbA1c and blood sugar were carried out at Diabetic research laboratory, Tertiary care centre, Teaching Institute. 110 Non diabetic persons studied as a control, which were proved to be Non diabetic from history, FBS, PPBS, Urine sugar. Persons with family history of diabetes were not included in control group **(Group: X)**. 350 diabetic patients which included new and old cases, IDDM and NIDDM cases, complicated and non complicated cases, among them 241 were having NIDDM and 109 were having IDDM type of diabetes **(Group: Y+Z)**. All cases thoroughly studied and details about personal data, history, clinical examination, laboratory investigations, complication of diabetes and type of treatment were noted.

Results: In IDDM, there was higher value of mean GHb (13.13%), than in NIDDM (mean GHB 11.89%). Patients having Insulin therapy had higher value of GHb (13.08%) than with on oral hypoglycemic agents (11.91%) and patients on dietary modification had level 9.44%. There was no

significant difference in GHb among patients with complication (12.26%) and patients without complications (12.29%).

Conclusion: Glycosylated hemoglobin assay defines an end point as the fuel of diabetic therapy and provides a powerful stimulus to the patients to improve their compliance. Glycosylated hemoglobin assay may provide an alternative method of screening for diabetes.

Key words

Glycosylated hemoglobin, Diabetes, Diagnostic test, Prognostic test, IDDM, NIDDM.

Introduction

Both science and the practice of medicine change rapidly. In recent year, new developments have taken place of breath taking speed. The most important of these is the science pertaining to health and disease. One of the new concepts is that careful control of blood glucose helps to prevent or postpone the dread complications of angioplasty and neuropathy in the course of diabetes Mellitus. A deterrent to greater acceptance of this is lack of control of blood sugar. Prospective studies suggest that there is positive relationship between the degree of metabolic control and the frequency and extent of late complications of Diabetes mellitus [1].

Investigation of the structure and biosynthesis of glycosylated Hemoglobin (HbA1c) in the past decade have provided a means to objectively access the average level of glycemia in diabetic patient. The use of Glycosylated hemoglobin level as integrated index of long term blood glucose level, represent a significant tool in our research and therapeutic armamentarium [2].

From the structural and biosynthesis information available it is clear that HbA1c is formed slowly and almost irreversibly by the condensation of glucose and hemoglobin in red blood cell. At any given point of time glycosylated hemoglobin level is better indicator of blood glucose. The process of glycosylation is continuous through whole 120 day life span of red blood cells, so it correlates with glucose levels of previous 6 to 8 weeks [3].

In this study, we have estimated glycosylated Hemoglobin (HbA1c) in diabetic and non diabetic person and its relationship with fasting and post prandial blood sugar levels.

By studying the patients with apparently well controlled diabetes as indicated by their FBS, FUS, PPBS and PPUS levels an attempt has been made to judge the actual control of diabetes using glycosylated Hemoglobin (HbA1c).

Aim and objectives

- To study significance of glycolsylated hemoglobin in diabetic patients in comparison with normal control person.
- To find out role of glycolsylated hemoglobin in diabetic patients as a diagnostic and prognostic test.
- To compare diagnostic value of glycolsylated hemoglobin with routinely used Fasting and Postprandial blood sugar values.
- To study difference of levels of glycolsylated hemoglobin in well controlled and poorly controlled diabetic patients.
- To study difference of levels of glycolsylated hemoglobin in male and female diabetic patients.
- To study difference of levels of glycolsylated hemoglobin in Insulin dependent diabetes mellitus and Non insulin dependent diabetes mellitus.
- To study difference of levels of glycolsylated hemoglobin in diabetic patients who have long term complications of diabetes mellitus and those who do not have long term complications.

- To find out difference of level of glycolsylated hemoglobin in obese and non obese patients
- To find out difference of level of glycolsylated hemoglobin in diabetic patients having different modes of treatment.
- To find out difference of level of glycolsylated hemoglobin in diabetic patients having positive Family history of diabetes and those who do not have family history.
- To find out help of glycolsylated hemoglobin assay, in diabetic patients as a good tool of measuring glycemic control.

Material and methods

In present study, Glycosylated hemoglobin levels were estimated by using cation exchange resin method. The study was conducted from November 2012 to October 2014. Measurement of total HbA1c and blood sugar were carried out at Diabetic research laboratory, Tertiary care centre, Teaching Institute.

110 Non diabetic persons studied as a control, which were proved to be Non diabetic from history, FBS, PPBS, Urine sugar. Persons with family history of diabetes were not included in control group **(Group: X)**

350 diabetic patients which included new and old cases, IDDM and NIDDM cases, complicated and non complicated cases, among them 241 were having NIDDM and 109 were having IDDM type of diabetes **(Group: Y+Z)**

All patients were selected from the cases who were admitted in tertiary care centre or attending diabetic clinics as outdoor patients.

All cases thoroughly studied and details about personal data, history, clinical examination, laboratory investigations, complication of diabetes and type of treatment were noted.

In all cases Glycosyalted hemoglobin measurement were done on the same day of fasting and post prandial blood sugar estimation. Collection of sample was done along with FBS or PPBS, in E.D.T.A. vaccute. Determination of GHb was done by ion exchange resin method and blood sugar estimation was done by chemical method with 100 mg% glucose standard.

Results

In this study, 110 non diabetic control subjects and 350 diabetic patients were studied. In study group of 350 diabetic patients there were Type-I, Type-II, Newly diagnosed as well as established cases, well controlled and poorly controlled cases, patients with and without complications. Throughout this study, results of different parameters were expressed by using these groups.

Control group

Control group included 110 non diabetic normal subjects. Out of these 110 normal subjects, 66 were male and 44 were females, mean age of them was 40.63 years. (Range: 18-70 years) Mean HbA1c value in this group was 6.66. Range was 5.7 to 7.8 (**Table – 1**).

Study group (Y+Z)

These included all 350 diabetic patients and of these 220 were male and 130 were females. Mean age of them was 44.14 (Range- 14-75 years).

Table – 1: Control group **(Group = X)**

Group (X)		No.	%	FBS (mean)	PPBS (mean)	GHB (mean)
Control	**T**	110	100	95.91	135.09	6.66
	M	66	60	96.65	134.01	6.71
	F	44	40	94.81	136.5	6.59

In this group two subgroups were identified.

Group Y: (well controlled diabetic) which was defined as blood sugar in non diabetic range: FBS <120 mg/dl and PPBS <180 mg/dl. In this 40 patients were included, and out of these 26 were male and 14 were females.

Group Z: (poorly controlled diabetic) which was defined as blood sugar in diabetic range: FBS >120 mg/dl and PPBS >180 mg/dl.

In this 310 patients were included, and out of this 194 were male and 116 were female. All those patients, who had duration of diabetes within three months from the day of measurement were considered "newly detected" diabetic patients and rest of the patients were considered "old case" or "established case". There were 47 newly detected and 303 old cases of diabetes in this study using above criteria. In the study group (Y+Z) there was mean HbA1c value is 12.28. The lowest value being 7.1 and the highest value is 15.5. The mean value was almost being two times higher than that control group (**Table – 2**).

Table - 2: Case group.

Group	GHb%	
	Mean ± SD	Range
Y	8.94 ± 1.04	7.1 to 12.42
Z	12.71 ± 1.84	9.22 to 15.5

Age distribution

In case group mean age was 44.14 years and in this group the maximum number of patients were in 41 to 50 year age group. The minimum age being 14 years and maximum age was 75 years (**Table – 3**).

Table - 3: Age distribution.

Group	No.	%	GHb (mean)
Case	350	100	12.28
10-20 years	23	6.57	12.61
21-30 years	55	15.72	12.78
31-40 years	47	13.42	12.38
41-50 years	101	28.85	12.37
51-60 years	81	23.15	11.88
61-73 years	43	12.29	11.86

BMI and sex wise distribution

In study group the mean BMI was 23.55 kg/m^2. In male mean BMI was 23.92 kg/m^2 and in female 22.91 kg/m^2. Mean BMI was higher in male than in female. Minimum BMI was 18.4 kg/m^2 while maximum BMI was 45 kg/m^2 (**Table – 4**).

Table - 4: BMI and sex wise distribution.

Group	No.	%	BMI Kg/m^2 (mean)	GHb (mean)
Case	350	100	23.55	12.28
Male	220	62.85	23.92	12.10
Female	130	37.15	22.91	12.57

There were 45 overweight or obese patients in this study group (Average BMI: 23.55 kg/m^2). Obese patients showed significantly higher GHb levels. Mean GHb level was13.64%. In this study, mean GHb level of female was 12.57% which was more than GHb level of males (12.10%) (**Table – 5**).

Table - 5: Obesity and GHb.

Group	No.	%	GHb (mean)
Case	350	100	12.28
Non-obese	305	87.14	12.08
Obese	45	12.86	13.64

Type of diabetes mellitus

In control group mean GHb of male was 6.71 and female 6.59, in study group mean GHb level was 12.28%, in male 12.10% and in female 12.57%. In IDDM, mean GHb was 13.13%, male had GHb level 13.03% and in female 13.23%. In NIDDM, mean GHb was 11.89%, male had GHb level 11.77% and female had 12.14%, having higher value in female than in male. The clinical diagnosis of Type I (IDDM) versus Type II (NIDDM) diabetes mellitus was established at the time of measurement of GHb according to the following criteria:

- Age at the onset of diabetes
- Rapid or slow onset

- History of significant ketonuria and/or body weight

In IDDM, the mean GHB was 13.13%, mean age was 42 years (range- 15-69). In NIDDM, The mean GHB was 11.89%, mean age was 45 years (range- 14-75). Thus, in IDDM there was higher value of mean GHB 13.13%, than in NIDDM (mean GHB 11.89%) as per **Table – 6**.

Table - 6: Type of Diabetes Mellitus.

Group	No.	%	GHb (mean)
Control	110	100	6.66
Case	350	100	12.28
IDDM	109	31.14	13.13
NIDDM	241	68.86	11.89

Duration of Diabetes Mellitus

In study group as the duration of DM was increasing, the level of GHb also increased steadily as per **Table - 7**.

Table - 7: Duration of Diabetes Mellitus.

Group	No.	%	GHb (mean)
Case	350	100	12.28
0-5 years	194	55.43	11.94
5-10 years	86	24.57	12.57
>10 years	70	20	12.85

Study of family history of diabetes mellitus in study group

Out of 350 diabetic patients, family history of diabetes was positive in 87 patients, out of these 39 were of NIDDM, 48 of IDDM (**Table – 8**).

Table - 8: Family history of dm.

Group	No.	%	GHb (mean)
Case	350	100	12.28
With F/H	87	24.85	12.5
Without F/H	263	75.15	12.21

Complication of DM

In this case study group of 350 patients, there were 131 patients having long term complications of diabetes mellitus like peripheral neuropathy, diabetic retinopathy, peripheral vascular disease and ischemic heart disease.

There was no significant difference in patients with complication (12.26%) and patients without complications (12.29%) as per **Table - 9**.

Table - 9: Complication of DM.

Group	No.	%	GHb (mean)
Case	350	100	12.28
With complication	131	37.43	12.26
Without complication	219	62.57	12.29

Like diabetic neuropathy, the chronic progressive renal failure also results. Because of this red blood cell survival decreases, so there was decrease in GHb levels. In this study mean GHb level of patients with renal complication was 9.08% as per **Table - 10**.

Table - 10:- Renal disease and DM.

Group	No.	%	GHb (mean)
Case study	350	100	12.28
Case with complication	131	37.43	12.66
Case with renal complication	15	11.45	9.08

Type of treatment and GHb

These showed patients having Insulin therapy had higher value (13.08%) than with on oral hypoglycemic agents (11.91%) and patients on dietary modification had level 9.44% as per **Table - 11**.

Table - 11: Type of treatment and GHb level in present study.

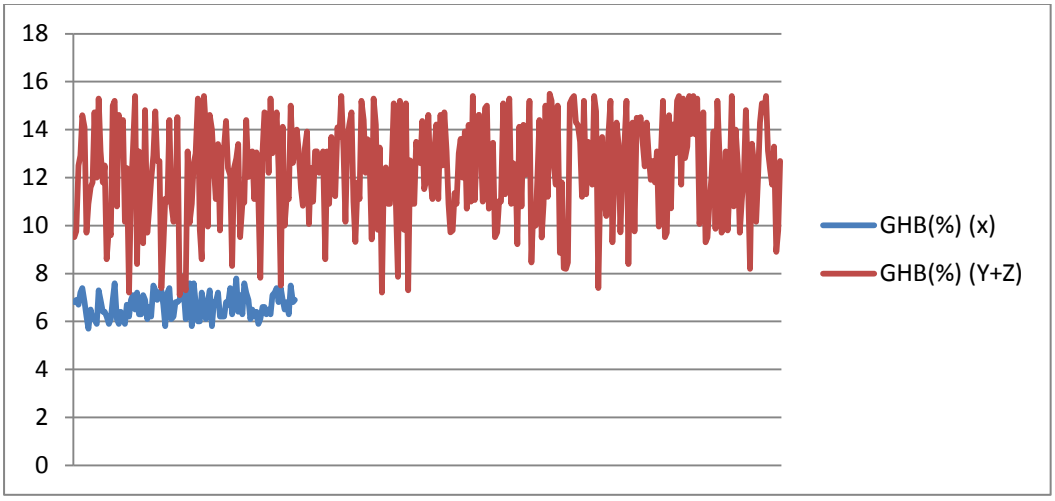
Type of treatment	No.	GHb (mean)
Insulin	136	13.08
Oral hypoglycemic agent	202	11.91
Dietary modification	12	9.44

Mean value of all three variables were significantly greater in diabetic group than in non diabetic group as per **Table - 12**. HBA1c, FBS, PPBS value for each group were plotted in **Graph - I(A), Graph - I(B) and Graph - I(C)** respectively. There was slight overlap of HBA1c in diabetic and non diabetic patients but marked overlap in FBS and PPBS in diabetic and non diabetic patients.

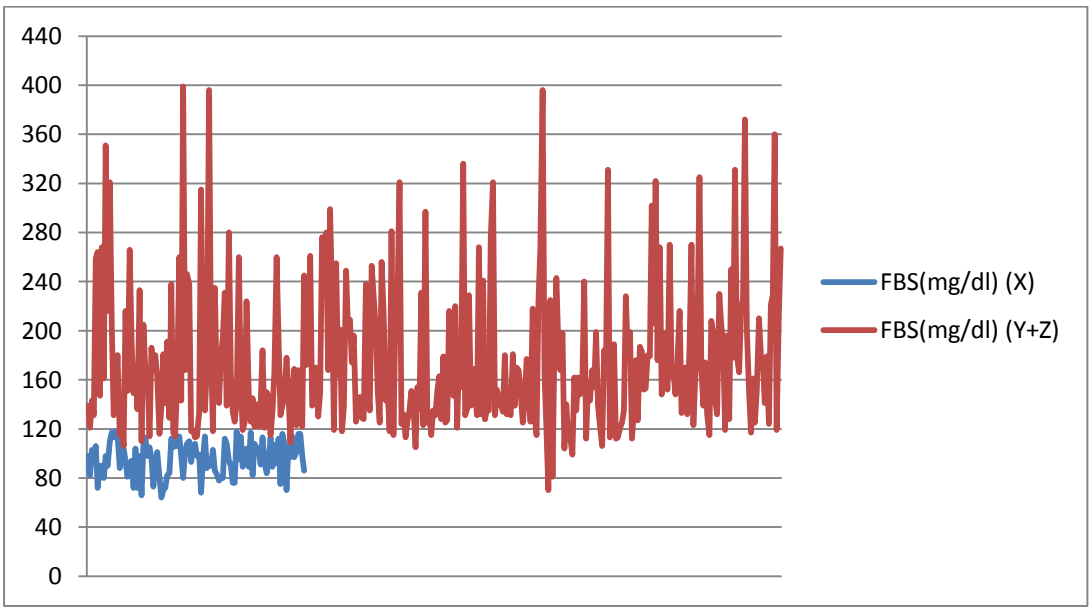
Table - 12: Various parameters of diabetes control and HBA1c.

Group	No.	FBS (mean)	PPBS (mean)	GHb (mean)
Control (X)	**110**	95.91	135.09	6.66
Study (Y+Z)	**350**	174.54	248.49	12.28
IDDM	**109**	175.10	245.44	13.13
NIDDM	**241**	184.29	258.64	11.89

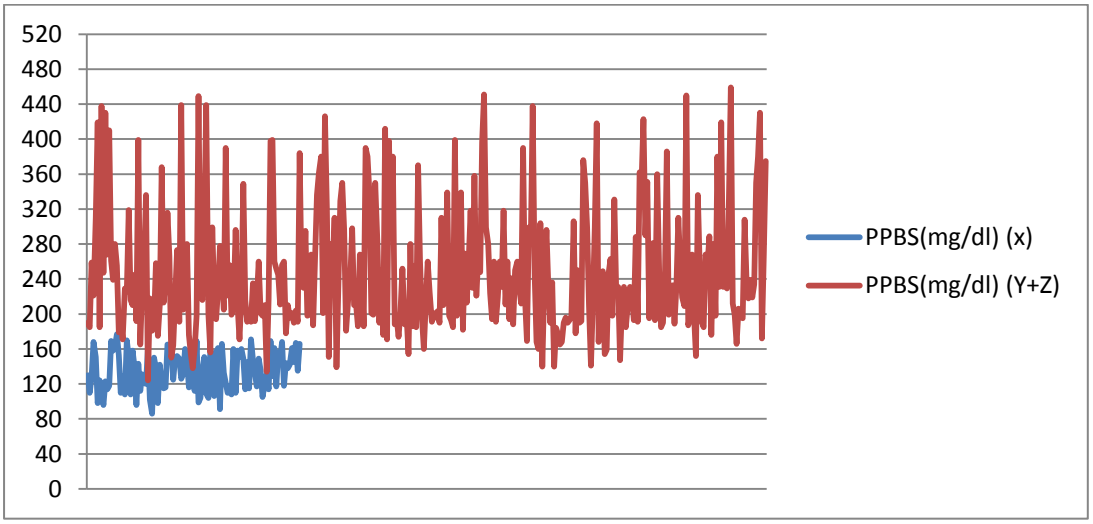
Graph - I (A): Comparison of HBA1c value between Diabetic and Non Diabetic patients.



Graph - I(B): Comparison of FBS value between Diabetic and Non Diabetic patients.



Graph - I(C): Comparison of PPBS value between Diabetic and Non Diabetic patients.



Discussion

The goal of prevention of long term diabetic complication may be achieved by chronic glycemic control in diabetic patients. To obtain this goal, proper assessment of glycemic control becomes an essential part of management. Determination of Glycosylated hemoglobin is new and unique method of measuring the level of chronic glycemic control in diabetic patients.

This study was conducted on **Control group (X)** of 110 non diabetic persons and **Study group (Y+Z)** of 350 diabetic patients to find relation of glycosylated hemoglobin and different aspects of diabetes mellitus. In this study various aspects of diabetes like age, type of diabetes, type of treatment, family history, complications and effect of these parameters on levels of Glycosylated hemoglobin was studied and levels of GHb measured by Ion exchange resin method.

In control group (non-diabetic, n=110) mean HbA1C was 6.66 and with standard deviation,(mean $\pm$ SD) 6.66 $\pm$ 0.78 (range from 5.7% to 7.8). These findings are in accordance with the previous reported range by S.K. Khullar (1984) [4].

In diabetic patients (n=350) mean HbA1c value was 12.28 % and range: mean +-SD: 12.28 +-2.52 (7.1 to 15.5), so this was also in accordance with the previous study by Abraham E.C. (1978) [5], S.K. Khullar (1984) [4].

This clearly reflected that HbA1c values in diabetic patients are 2 to 3 times higher than non-diabetic patients [4].

Higher glycosylated hemoglobin values were in younger age groups 10 to 40 years and levels were less than younger patients seen in age group of more than 40 years (41 to 75 years), Ronald Klein, et al. (1987) have selected that higher mean GHb were found younger age patients [1].

Mean value of GHb was high in female patients (12.57%) than mean values of male patients (12.10%) and also there was mean BMI for male patients was 23.92 kg/m^2 and mean BMI for female was 22.91 kg/m^2 which indicated value was higher in males. These type of result were also obtained by David E. Gold Stein and Stickland M.H. (1984). Stickland stated that because of chronic blood loss (menstruation) and sex difference in Red blood cell life span (109) days female hemoglobin can undergo greater degree of glycosylation compared to that of male,. That's why female diabetic patients have higher value higher of HbA1c [6, 7].

The levels of Glycosylated Hemoglobin in obese patients was significantly higher (13.64%) than

that the non-obese patients (12.08%) which correlated with Burn, et al. (1981) showed similar kind of result of elevated glycosyalted hemoglobin value in obese patients [8].

The levels of GHb were higher in Insulin dependent diabetes mellitus (IDDM, type-I, Juvenile) - mean 13.13% than in Non- Insulin dependent diabetes mellitus (NIDDM, type-II, maturity onset) – mean 11.89%. Similar type of result obtained by David M. et al. (1984) [9].

In this study, the level of GHb increases progressively as the duration of diabetes increase. Though patients having newly detected diabetes cases and in initial periods show increased levels of GHb. David Goldstein (1982) stated that generally longer the duration, higher will be the value of HbA1c [10].

Patents with long term complications of diabetes had mean GHb 12.26 % and patients without complications had mean GHb of 12.29%, so there was not much significant difference in levels of GHb between patients with complications or without complications. Koeing, et al. (1976) and Trivelli, et al. (1971) also have failed to establish such correlation [11, 12].

Patients on Insulin therapy had higher values of GHb (13.08%). Patients on oral hypoglycemic drugs had GHb level 11.91%, and patients on dietary medication had GHb level 9.44%. These finding were consistent with finding of David E. Goldstein, et al (1982) [10].

HbA1c determination may provide an alternative method of screening for diabetes. This is an attractive concept because HbA1c level can be measured from a single blood sample taken at any time of day without prior dietary preparation and yet provide highly representative measure of the average blood glucose concentrations as per P.J. Dunn, et al., (1979) [13]. Oral glucose tolerance test has poor reproducibility and results are affected by various factors. Moreover, it is non physiological test in nature. The load of 75 grams glucose is not encountered in day to day life as per S.K. Khullar (1984) [4]. Thus the oral glucose tolerance test, although a very sensitive test to diagnose patients with diabetes mellitus, has low specificity. In contrast the HbA1c determination has low sensitivity but high specificity as per P.J. Dunn, et al., (1979) [13]. The sensitivity is high if test is directly related to the quality of determination.

Even when 99.7% confidence limit was taken for normal range, there was overlap of HbA1c levels in 2 cases of control and diabetics, so we can say that subjects with HbA1c value less than 9.08% were likely to be non- diabetics. So, cut of point of 9.08% of HbA1c levels for normal and diabetic was suggested. These finding in present series confirmed the results of previous study done by S.K. Khullar (1984), that HbA1c can be used as screening procedure for detection of diabetics [4].

HbA1c level in group "Z" i.e. diabetics patients with poor control (i.e. blood sugar in diabetic range) is 12.71+-1.84 (range 9.22% to 15.5%) and this is highly significant than the values in control group in present study.

Diabetes mellitus, a common metabolic disorder, which accounts for a high incidence of morbidity leads to various events including micro and macro vascular complications [14-17]. Glycosylated hemoglobin assay is superior to the traditional blood sugar values in discriminating diabetic from non-diabetic patients. Graph-I(A), Graph-I(B) and Graph-I(C) show results of three parameter of diabetic control, HbA1c, FBS, PPBS, respectively, in non-diabetic and diabetic subject. There is overlap of the diabetic and non diabetic HbA1c value in 8 cases (2.28%) out 350 patients. Such an overlap in FBS value is also found in 32 cases (9.14%). Out of 350 patients, for post prandial blood sugar values such overlap is present in 36 out 350 patients (10.28%). These findings are consistent with generally accepted mechanism of formation of glycosylated hemoglobin, that it is an integral related to the average blood sugar to which the hemoglobin is exposed.

Consequently this should provide a better discriminate of diabetic from the non-diabetic than a rapidly fluctuating variable like blood sugar values.

The patients in Graph - I(A), Graph - I(B) and Graph - I(C) having values of HbA1c, FBS, PPBS in normal range, are considered as members of group Y (well controlled) .Some of the other patients also have their FBS and PPBS values in non diabetic range. Their HbA1c values also should be in non diabetic range, but instead they have higher values of HbA1c because HbA1c reflects average blood glucose control of previous several weeks. Thus HbA1c is able to isolate patients having overt hyperglycemia in previous weeks, while sugar levels reflect transient diabetic control at the time of measurement. This finding clearly shows superiority of HbA1c assay over blood sugar values in judging the control of diabetic patients.

Conclusion

Periodic monitoring of HbA1c should allow the assessment of chronic diabetic control on an out patients basis in a more objective manner than is now possible and enable one to evaluate various forms of therapy and the relationship between carbohydrate control and the progression of various diabetic sequel. Glycosylated hemoglobin assay provides a better discriminate of diabetic from the non diabetic than a rapidly fluctuating variable like blood sugar. Discrepancy between the results of glycosylated hemoglobin and results of blood sugar level of patients, can give an indication to treating physician to look back into detail history and modify the therapeutic regimen accordingly. Glycosylated hemoglobin assay defines an end point as the fuel of diabetic therapy and provides a powerful stimulus to the patients to improve their compliance. Glycosylated hemoglobin assay may provide an alternative method of screening for diabetes.

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