A Prospective Therapeutic Assessment of Diabetic Hyperglycemia by the Estimation of Glycated HBA,HbA1C, MBG

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ABSTRACT

Introduction: Glycosylated haemoglobin is a normal adult Hb (HbA1) which is covalently bound to glucose molecule. GHb concentration is depending on the average blood glucose concentration. GHb is now widely recognised as a reliable indicator of the efficiency of the therapy. Current study aimed to know the efficiency of treatment in the cases of type II diabetes.
Material and methods: the work is done by using Excels GHb kit based upon the property of non-Glycated Hb to bind with a weak cat ion exchange resin leading GHb free in the supernatant.
Results: out of 50 patients, 18(32%) were under poor control, alarming the regular monitoring of blood glucose levels.
Conclusion: Estimation of glycated haemoglobin during antidiabetic therapy helps the patient to know the therapeutic efficiency of the drug.

Keywords; HbA1C, hyperglycemia, type II diabetes, therapeutic assessment

INTRODUCTION

Hyperglycemia is the defining characteristic of the disease called DM and complications. Measurement of diabetic control (it is the extent to which metabolism in the diabetic patient differs from that in the non diabetic patient) is used for the diagnosis of diabetics for adjusting therapy so as to maintain near –normal blood concentration of glucose.[1] Glycated Hb (Glycohemoglobin, Glycosylated haemoglobin) is the corner stone of glycemic control in all major trials like DCCT in type I diabetes and UKPDS(United Kingdom Prospective Study) in type II diabetes.[2] There is a body of evidence that GHb relates to integrated preceding glycemic control.[3,4] According to the trials from DCCT and UKPDS HbA1C percentage can be regarded as a risk factor for the development of microangiopathy.[5] Blood glucose concentrations in the non-diabetic person are maintained within normal limits around 5m mol/lit (90mg/100ml) throught the day. The American diabetes association recommended the goals of diabetes therapy should be an HbA1C 7% and treatment should be modified when the values are consistently > 8%. Type II diabetes is not a mild disease. Chronic complications are common and potentially severe –notable retinopathy with maculopathy, nephropathy and coronary heart, cerebrovascular and peripheral arterial disease. Macroangiopathy related to heart is two to three times more in diabetics than in non-diabetics and up to 75% people dies from cardiovascular causes, so good control of glycemic haemoglobin (HbA1C) is mandatory in order to postpone the complications. Glycated hemglobin estimation determines the long term control of blood glucose levels when compared to single blood glucose estimation , which is effected by diet, insulin, exercise on the day of testing. GHb is now widely recognised as a reliable indicator of the efficiency of therapy. There is an excellent correlation between HbA1C concentration and diabetic control.[6] The first description of hemoglobin A1c (HbA1c) has been variously ascribed to Kunkel[7] or Huisman,[8] but Rahbar, in 1969, is generally credited with the recognition of HbA1c as abnormal in diabetes.[9] HbA1C is having correlation with mean blood glucose levels on comparison with other markers of glycemic control like glycated albumin.[10] Diabetes mellitus is a major health problem of increasing magnitude worldwide with a great impact on cardiovascular morbidity and mortality.[11] Hemoglobin A1c concentration appears to reflect the mean blood sugar concentration best over previous weeks to months. As HbA1C reflects the long term mean blood glucose levels, periodic estimation of it is helpful to know the long term metabolic control of blood glucose.[12]

Aim of the study was to assess the efficiency of treatment in type II diabetics during therapy with oral hypoglycaemic drugs in Government General Hospital.

MATERIAL AND METHODS

Study was conducted in Govt General Hospital, Guntur, during the period of May- 2016 to October -2016. In the present study 50 cases of type II diabetes mellitus were taken who were under treatment with oral hypoglycaemic drugs irrespective of sex. Samples were tested for the estimation of Glycated haemoglobin- MBG, HbA1C. Excell GHb kit based upon the property of non-glycated Hb to bind with a weak cat ion exchange resin, so that GHb can be estimated as it is separated. There were several acceptable methods of GHb measurements like electrophoresis, ion exchange chromatography, HPLC and calorimetry.

Estimation of sample

Step I: hemolysate preparation

Whole blood was collected by phlebotomy. A volume of 0.05ml (50µl) taken and mixed with 0.25ml (250µl) lysing reagent to prepare a hemolysate for 5mts.

2. Step II

0.1ml was taken from the hemolysate and added to the tube containing cat ion exchange resin.

Positioned resin separator in the tube so that the rubber sleeve is 3cm above the resin. Mixed the contents in the vertex mixture

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continuously for 5mts.
Allowed the resin to settle at assay temperature for 5mts.Pushed down the resin separator in the tube until the resin is firmly packed, and measured the absorbance (A) against deionised water.

Step -III

0.02ml (20µl) of hemolysate taken in tube containing 5.0ml of deionised water, mixed and read absorbance against deionised water.
The non-glycated Hb binds to the resin, leaving GHb free in the supernatant. The GHb percentage was determined by measuring the absorbance of the GHb fraction and of the total Hb.

Calculation

GHb%=A of GHb/A of THbX10XTemp. factor (Tf)
Assay can be done at 23ºC for which temperature factor (Tf) is one.
Glycosylated haemoglobin HbA1C was formed spontaneously in red blood cells by combination of NH2 terminal groups of the haemoglobin B chain and glucose. The aldehyde group of glucose first forms a Schiff base with the NH2-terminal amino group. This then rearranged to a more stable amino ketone linkage, by a spontaneous (non enzymatic reaction) reaction known as the amadori re arrangement**.**
The concentration of HbA1C is dependent on the concentration of glucose in the blood and the duration of hyperglycemia. In prolonged hyperglycemia the concentration may rise to 12% or more of the total haemoglobin. Patients with DM have high concentration of blood glucose and therefore high amounts of HbA1C.

S. No	HbA-A1	MBG	HbA1C
1	8.0	141.6	6.55
2	7.9	139	6.48
3	7.4	130	6.15
4	7.1	124	5.95
5	7.5	132	6.21
6	7.7	135	6.35
7	5.0	84	4.54
8	7.8	137	6.42
9	7.9	139	6.48
10	5.7	97	5.01

Table-1: Patients under normal control

S. No	GHb-A1	MBG	HbA1C
1	8.0	141.6	6.55
2	9.0	160	7.22
3	9.0	160	7.22
4	8.5	151	6.89
5	8.6	153	6.95
6	8.5	151	6.89
7	8.1	143	6.62
8	8.1	143	6.62
9	8.7	155	7.02
10	8.6	153	6.95
11	9.0	160	7.22
12	8.7	155	7.02
13	8.5	167	6.89
14	8.9	158	7.15

Table-2: Good control

RESULTS

Interpretation of results is based on the following ranges of glycated haemoglobin (GHb %)
Normal range: 4.5-8%
Good control: 8-9%
Fair control: 9-10%
Poor control: 10 and above 10%
Among 50 cases 10 patients (20%) shows glycated haemoglobin (HbA1) within normal range (Table-1), 14(28%) members showed glycated haemoglobin (HbA1) in between 8-9 indicating good control (Table-2), and 8(16%) cases are under fair control (Table-3) i.e. glycated haemoglobin (HbA1) is in between 9-10. Poor control was shown by 18(32%) patients (Table-4).
Out of 50 patients 10 patients showed HbA1c levels between 4.54-6.55 indicating normal range, 14 members having HbA1C levels between 6.55-7.22 i.e. they are having good control of diabetes. HbA1C levels are between 7.22 -7.89 among 8 members showing fair control. Out of 50 patients 18 members showed HbA1C levels more than 10 indicating poor control (Table-5).
Among 50 patients 10 cases showed MBG between 84-141mg%, whereas 14 patients were having levels between 141-160mg%. Fair control was shown by 8 patients with MBG levels 160-180mg% and poor control by 18 patients with MBG levels more than 180mg%.

DISCUSSION

According to recent statistical analysis there are about 40.9

S. No	GHb-A1	MBG	HbA1C
1	9.3	166	7.42
2	9.3	166	7.42
3	9.2	164	7.36
4	9.8	176	7.76
5	9.3	166	7.42
6	9.2	164	7.36
7	9.3	166	7.42
8	9.3	166	7.42

Table-3: Fair control

S.no	HbA-A1	MBG	HbA1C
1	11.67	210	8.9
2	11.1	201	8.63
3	15.6	287	11.65
4	10.6	191	8.29
5	11.6	210	8.97
6	16.4	302	12.19
7	11.0	199	8.56
8	12.3	224	9.44
9	17.8	329	13.13
10	10.2	183	8.03
11	14.0	256	10.58
12	13.9	254	10.51
13	15.6	287	11.65
14	10.7	193	8.36
15	23	429	16.62
16	8.2	337	13.39
17	12.2	222	9.37
18	12.3	224	9.44

Table-4: Poor control

Parameter	Normal (20)%	Good control (28%)	Fair control (16%)	Poor control (32%)
Glycated Hb	4.5-8%	8-9%	9-10	More than 10
HbA1C	4.54-6.55	6.55-7.22	7.22-7.89	More than 7.89
MBG	84-141mg%	141-160mg%	160-180mg%	More than 180mg%

Table-5: Showing normal ranges of individual parameters with patients diabetic control in percentages

million cases of diabetes and it may rise up to 69.9 million by the end of 2025 if proper care is not taken.[13]

Assessment of HbA1C is more convenient and advantageous as it does not require fasting and no pre analytical variations.[14,15] Improvement in HbA1C levels were also reported in a study done by Bastyr et al showed that the therapeutic lowering of postprandial blood glucose and fasting blood glucose levels leads to lowering of HbA1C.[16]

Another study showed there is lack of strong correlation between HbA1C and glucose levels in a single day, but there is a strong correlation between HbA1C and mean daily glucose levels.[17]

In the present study 50 cases of Diabetes mellitus (type-2) were studied irrespective of sex to know the efficiency of treatment with oral hypoglycaemic agents by the estimation of glycated haemoglobin (HbA1), MBG (mean blood glucose), and HbA1C. Using the conversion chart of glycosylated haemoglobin A1% mean blood glucose and HbA1C are derived. HbA1 essay is a reliable indicator of mean blood glucose (MBG). In the present study 32% of patients showed poor control, it indicates that even though the patients are under treatment they were unable to maintain controlled sugar levels. Regular monitoring of HbA1C helps in therapeutic assessment of patient.

Abraham et al.[6] reported excellent correlation between HbA1 concentration and diabetic control and concluded that the determination of HbA1 rather than HbA1C (a fraction of HbA1) be used for clinical purpose.

CONCLUSION

HbA1 is now widely recognised as a reliable indicator of the efficiency of therapy. It reflects long term metabolic control of glucose in individuals. In the present study we observed blood glucose levels are not maintaining properly even after treatment. So estimation of HbA1, MBG, and HbA1C will help the patient for therapeutic assessment of their long term glucose levels in order to avoid long term diabetic complications like macro angiopathy, diabetic retinopathy, and diabetic nephropathy.

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