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Original Research Article

A comparative evaluation of blood sugar and glycosylated hemoglobin in clinically manifested diabetic neuropathy

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ABSTRACT

Background: It is very well established that tight control of diabetes reduces if not prevents the risk of neuropathy. The benefit of other mode of therapy like myo- inositol supplementation and aldose reductase inhibitors remain to be established. Objective of present study to compare blood sugar and glycosylated hemoglobin in clinically manifested diabetic neuropathy.
Methods: Hospital based cross sectional study was carried out at Department of General Medicine, from August 2016 to October 2017 among 60 patients of diabetic neuropathy. These patients were subjected thorough evaluation as per the proforma.
Results: As per the blood sugar levels, 15% were normal. As per glycosylated hemoglobin levels, only one patient was found to be normal. Thus, glycosylated hemoglobin showed evidence of poor control more frequently than blood sugar estimation in these patients. Patients with both retinopathy and neuropathy in this study had diabetes mellitus for periods 2 months to 20 years. (Mean 8.2yrs). Thus, it can be seen that glycosylated hemoglobin is a more sensitive indicator of poor control of diabetes mellitus than blood sugar. This difference is statistically significant (P <0.05).
Conclusions: Thus, as an integral of diabetic control, glycosylated hemoglobin (HbA1C) estimation is superior to the conventional measures in assessment of control.

Keywords: Blood sugar, Diabetes, HbA1C

INTRODUCTION

The introduction of Insulin in 1922 by Banting and Best seemed to offer the Ideal therapy for the treatment of diabetes mellitus, replacement of missing hormone, indeed in the past 50 year the Judicious use of Insulin has made it possible control the symptoms of hyperglycemia and to avoid death from Ketoacidosis. However, despite the continual use of Insulin, many pathological changes (e.g. Retinopathy, Angiopathy, Nephropathy and Neuropathy), still develop and now account for the major morbidity and mortality associated with the disease.[1]

One of the main difficulties to establish whether there is a relation between degree of Hyperglycemia and long term complication of diabetes is the lack of reliable and objective method for assessing diabetic control. The clinician at present has no quick and simple way of ascertaining. Whether modification of therapeutic regimen has altered control for better and worse. Blood and urine glucose testing and urine ketone testing provide useful information for day to day management of diabetes. However, these tests cannot provide the patient and health care team with a quantitative and reliable measure of glycemia over-an extended period of time. And these tests have drawback of demands patient

compliance or frequent measurement. Measurements of glycated proteins, primary hemoglobin and serum proteins, have added a new dimension to assessment of glycemic. With single measurement of each of these tests, can quantity average glycemic over weeks and months, there by complementing day to day testing. Expert opinion recommends A1c testing at least two times a year in patients who have stable glycemic control.[2]

Lately Keontg and Gabbay and their co-workers have suggested measurement of glycosylated hemoglobin (HbA1C) as an indicator of diabetic control. HbA1C is formed by the post-transcriptional glycosylation of HbA at the amino-terminal valine of beta chain. This is a slow irreversible chemical reaction which occurs throughout the life span of the RBC, the prevailing plasma-glucose concentration being the most important factor governing the quantity of HbA1C formed.

HbA1C can be separated from the major hemoglobin fraction by virtue of its fast movement through a cation exchange resin. When properly assayed HbA1C level in a blood sample gives an estimate of diabetic control for earlier 3-4 month period (i.e. life span of RBC).[1,2]

Diabetic neuropathy has been defined by the consensus conference of San Antonio as peripheral neuropathy either clinically evident or sub-clinically that occurs in the setting of diabetes mellitus without other causes.[3] The presence combination of the triad of neuropathy, retinopathy and nephropathy in the course of the lifelong disease regarded this "Triopathy" as consequences rather than complication.[4] Diabetic neuropathy is one of most common long term complication of diabetes mellitus and is clinically present in 30-50% of all diabetes patients.[5,6]

The present study has been undertaken to monitor the levels of blood sugar and HbA_{1C} in diabetic neuropathy. The study of diabetic neuropathy has been undertaken for the many reasons. The diabetes is a frequent cause of peripheral neuropathy. It affects almost every part of nervous system and produces, various type of neuropathy. It has significant morbidity and mortality. Its incidence increases, when the control of diabetes is poor.[7]

It is very well established that tight control of diabetes reduces if not prevents the risk of neuropathy. The benefit of other mode of therapy like myo- inositol supplementation and aldose reductase inhibitors remain to be established. Until then the clinician should monitor the patient's neurological status by routine methods and assess the control of diabetes by the available parameters and give practical advice that may save a limb and life.

METHODS

Hospital based cross sectional study was carried out at Department of General Medicine, from August 2016 to October 2017 among 60 patients of diabetic neuropathy. These patients were subjected thorough evaluation as per the proforma.

Inclusion criteria

- Loss of knee/ankle jerk
- Sensory deficits and
- Other neurological abnormalities

Exclusion criteria

- Other causes of neuropathy especially alcoholism
- Generalized areflexia without signs of neuropathy and
- Unilateral reflex loss

Among those diagnosed to be suffering from diabetic neuropathy, further exclusion of the factors which would lead to falsely abnormal, values for HBA1C was done before proceeding further.

- Anemia (Hb <10 gm%)
- Acute metabolic complications
- Ingestion of antibiotics and aspirin
- Hyperlipidemia

In all, 60 patients of diabetic neuropathy who satisfied the above criteria were selected and were subjected to a through evaluation as per working proforma.

Laboratory investigations done in all patients include

- Urine - Sugar, Albumin, Ketone bodies, Microscopy
- FBS and PPBS (Folin-wu method)
- Blood - Hb%, Urea, Creatinine, Cholesterol
- Glycosylated hemoglobin (HbA1C) by Ion Exchanges Chromatographic method.
- ECG, X-ray chest and other investigations whenever necessary were done. Hb A1C was estimated in blood sample taken for FBS estimation

Ion exchange Resin Chromatographic method of estimation of Hb A1C (GlycoHb) (Kynock and Lehmann 1977)

It is a rapid and simple method; total time required is less than 30 minutes.

Principle

Whole blood is mixed with a lysing reagent to prepare a hemolysate. This is then mixed with a weakly binding cation exchange resin.

The non-glycosylated hemoglobin binds to resin leaving Glycosylated Hemoglobin (HbA1C) free in the supernatant. The HbA1C is determined by measuring the absorbance of the HbA1C fraction and of the total Hb.

Reagents and apparatus

- Ion exchange resin (Bio-Rex 70)
- Hemolysing reagent: 0.3 g white saponin, 0.5 g potassium cyanide dissolved in a Buffer pH 6.7 to make 1 liter
- Control (lyophilized).
- Apparatus: plastic tubes and resin separators.

Specimen

Whole blood collected in EDTA bulb. Heparin may also be used. HbA1C in blood is found to be stable for one week at 2-8°C.

Equipment required were spectrophotometer/photo colorimeter, cuvettes, test tubes, vortex mixer, pipettes and micropipette.

Reagent preparation

Reagents 1 and 2 are ready to use. HbA1C control (3) is dissolved in 1 ml. of deionized water by inverting / swirling. Reconstituted control is stable for 30 min only at room temp or 15 days at -20°C.

Procedure

Assay temperature was 23±2°C, Wave length at 415 nm (Hg 405 nm)

Step 1: Hemolysate preparation

- O.5ml of lysing reagent (2) was pipetted into a test tube.
- To it 0.1ml of well mixed whole blood sample was added.
- Mixed and allowed to stand at Room Temperature for 5 minutes.

Step 2: Hb A1C separation and assay

- 3.0 ml of Ion Exchange Resin (1) was pipetted into the plastic tube and mixed well before use.
- 1.0ml of the hemolysate was added (from step 1)
- The resin separator was positioned in the plastic tube so that the rubber sleeve was approximately 2 cm above the liquid level.
- Plastic tube was placed on cortex mixer and was mixed for 5 minutes.
- The resin separator was pushed down in the plastic tube until the resin was firmly packed.
- The supernatant was poured directly into a cuvette and absorbance was measured against deionized water within 60 minutes.

Step: 3 total hemoglobin (THB) assay

- 5.0 ml of deionized water was pipetted into test tube.
- 0.02 ml of hemolysate (from step 1) was pipetted into it.
- Mixed and absorbance was read against deionized water within 60 minutes.

RESULTS

As per the blood sugar levels, 15% were normal, 23.3% were having good control, 41.6% had fair control, and 20% had poor control.

Table 1: Distribution of study subjects as per the degree of control of blood sugar.

Degree of control	Number	Percentage
Normal	9	15
Good control	14	23.3
Fair control	25	41.6
Poor control	12	20
Total	60	100

As per glycosylated hemoglobin levels, only one patient was found to be normal, 10% had good control, 23% had fair control and 65% had poor control of diabetes.

Table 2: Distribution of cases according to degree of control based on glycosylated hemoglobin.

Degree of control	Number	%
Normal (4.5-8%)	1	1.9
Good control (8-9%)	6	10
Fair control (9-10%)	14	23
Poor control (>10%)	39	65
Total	60	100

Thus, glycosylated hemoglobin showed evidence of poor control more frequently than blood sugar estimation in these patients.

The difference between this parameter as measures of poor control of diabetic was statistically significant.

Table 3: Association between blood sugar and glycosylated hemoglobin.

Degree of control	Blood sugar	Glycosylated hemoglobin
Normal	9 (15%)	1 (1.9%)
Good control	14 (23.3%)	6 (20%)
Fair control	25 (41.6%)	14 (23%)
Poor control	12 (20%)	39 (68%)

$X^2 = 26.99$, $p < 0.0001$

When these patients were evaluated for their diabetic control status depending on the presence of symptoms of diabetes, regularity or otherwise of the treatment, history of previous hospitalization for the complications, only 8 patients were judged to be under good control.

Table 4: Relation between blood sugar and glycosylated hemoglobin in patients with good control.

Degree of control	Blood sugar	Glycosylated hemoglobin
Normal	3	0
Good control	2	1
Fair control	3	1
Poor control	0	6

Table 5: Relation between blood sugar and glycosylated hemoglobin in patients with poor control.

Degree of control	Blood sugar	Glycosylated hemoglobin
Normal	7	1
Good control	14	3
Fair control	18	12
Poor control	7	30

$X^2 = 27.11$, $p < 0.0001$

When 46 patients, thought to be 'poorly' controlled diabetics using the same criteria, were further analyzed taking blood sugar and glycosylated hemoglobin criteria into consideration, following observation were obtained.

Table 6: Relation between blood sugar and glycosylated hemoglobin in patients with retinopathy and neuropathy.

Degree of control	Blood sugar	Glycosylated hemoglobin
Normal	7	1
Good control	14	3
Fair control	18	12
Poor control	7	30

$X^2 = 10.56$, $p < 0.05$

Patients with both retinopathy and neuropathy in this study had diabetes mellitus for periods 2 months to 20 years (Mean 8.2yrs). Whereas, patients with neuropathy alone had diabetes mellitus which was either detected on admission or was there for periods up to 10 years (Mean 8.2yrs). Thus, it is clear that longer the duration of diabetes, more are the chance for the development of complications of diabetes. Thus, it can be seen that glycosylated hemoglobin is a more sensitive indicator of poor control of diabetes mellitus than blood sugar. This difference is statistically significant ($P < 0.05$).

Table 7: Relation between blood sugar and glycosylated hemoglobin in patients with autonomic neuropathy.

Degree of control	Blood sugar	Glycosylated hemoglobin
Normal	3	0
Good control	3	5
Fair control	10	2
Poor control	2	11

18 patients had abnormal autonomic nervous system function as per the criteria laid down by Ewing and Clarke and two patients showed evidence of diabetic gastroparesis. Out of these 18 only 2 (11%) patients had blood sugar in the 'poorly' controlled category as compared to 11 (61%) patients in whom the glycosylated hemoglobin showed evidence of poor control. This difference was statically significant. After establishing the efficacy of the estimation of glycosylated hemoglobin the influence of the other parameters like, age, sex, duration and mode of therapy on its estimation was analyzed. The following observations were made.

Table 8: Duration of diabetes and control level of HbA1C.

Duration of diabetes (years)	Estimation of glycosylated hemoglobin				Total
	4.5-8%	8-9%	9-10%	>10%	
<5	0	3	2	13	18
6-10	1	2	11	10	24
11-15	0	1	1	12	14
>15	0	0	0	4	4
Total	1	6	14	39	60

42 patients had diabetes of less than 10 years duration, of which 23 patients had evidence of poor control of diabetes. Similarly 16 out of 18 patients with diabetes of more than 10 years duration had evidence of poor control the difference was not statistically significant ($P > 0.05$).

DISCUSSION

When properly assayed, the percent of Hb A1C provides a good retrospective, cumulative index of glycemic control for the preceding 3 months period.

Relation between HbA1C to Diabetic Hyperglycemia is derived from structural and biosynthetic information available this is clear that HbA_{1C} is formed slowly and almost irreversibly by the condensation of glucose and Hb in RBC.[8] With simultaneous accumulation of HbA_{1C} it is evident that the amount of this component should be a reflection of average glucose concentration seen by the RBCs during their life span.

Direct evidence for this relationship derives from at least three lines of evidence which include a reduction of Hb A_{1C} levels after diabetic patients are brought under optimal blood glucose control. A plethora of studies which demonstrate a relationship between Hb A_{1C} levels and a variety of indices of diabetic glycemia and excellent correlations between clinical evaluation of the patients level of control and Hb A_{1C} level.

Ellenbergh emphasized the fact that the neurological signs may already be present at the time of detection of diabetes itself.[9] This fact brought out in the present

study that 6 (10%) cases presented with Neuropathic symptoms as initial manifestation of diabetes.

Like American Diabetic Association (1988) blood sugar level, the present study documented the glycemic control in 4 levels which were measured by blood sugar and Glycosylated Hb level viz. Normal, Good, Fair and Poor.

It was observed that glycosylated hemoglobin level is the better indicator of poor glycemic control than blood sugar level (39 Vs 12) and statistically significant (P <0.001).

Further it was noted that in 8 patients who were initially thought to have good diabetic control prior to the hospital admission for neuropathy, 6 patients had raised glycosylated hemoglobin which indicated poor control. But the blood sugar did not reflect this fact.

Even in those patients who were thought to have poor metabolic control, levels of glycosylated hemoglobin were more consistent in indicating poor metabolic control than blood sugar levels. This difference was statistically significant (P <0.01).

Poor metabolic control with associated micro vascular complications like retinopathy was better reflected by GlycoHb level than blood sugar. The same result was observed Vercoe.[10]

Similar results were obtained in those patients who had objective evidence of autonomic neuropathy. This correlates with the study of Young et al who also documented deterioration of cardiovascular automic dysfunction with poor control of diabetes.[11]

Thus, it can be concluded that estimation of glycosylated hemoglobin is not only a simple rapid and objective procedure to assess diabetics control Boas Gonen et al but also a more sensitive and reliable indicator in monitoring the control.[12]

Matsumoto T et al showed the fasting blood sugar is major determinant of neuropathy independent of age, body mass index, and duration of diabetes.[13] The fasting hyperglycemia was observed in 83.3% (50) of patients; while post- prandial hyperglycemia was observe in 33.3% of patients in the study.

As Perice D et al showed erectile dysfunction was a common complication observed in diabetic men and was related to the may other complication, Sexual dysfunction was observed in 72.2% of diabetic men in study (26 out of 36 diabetic male patients).[14] The duration of diabetes was longer in these patients and the estimated glycosylated hemoglobin clearly showed poor controlled state.

Elevated levels of glycosylated hemoglobin were observed in most of the patients of diabetic neuropathy in the present study which supports the above outcomes.

The effect of various other parameters on the efficacy of glycosylated hemoglobin estimation as an indicator of poor metabolic control was also analyzed.

It was noted that the increased level of glycosylated hemoglobin did not appear to be related to the age, sex, duration of diabetes and mode of therapy. Thus, these factors do not come in the way of this procedure in assessment of the state of diabetic control. Similar observations have been observed by Trivelli et al.[15]

During optimal diabetic control the blood sugar concentration was 84 mg per deciliter (range, 70 to 100), and hemoglobin A1C concentration 5.8 per cent (range, 4.2 to 7.6). Hemoglobin A1C concentration appears to reflect the mean blood sugar concentration best over previous weeks to months.

Whereas in the present study it has been demonstrated that the sequelae of diabetes mellitus especially diabetic neuropathy has been the cornerstone for this study and thus the study indicated that Hba1c levels are directly related to the management of diabetic neuropathy.

Natural Progression of Diabetic Peripheral Neuropathy in the Zenarestat Study Population by Brown MJ and Bird SJ et al to report the baseline and natural progression of diabetic peripheral neuropathy over 12 months in a large mild-to-moderate neuropathy population concluded that neurologic decline over 12 months is evident when measured by nerve conduction studies and cool thermal quantitative sensory testing.[16] Other measures vibration QST, neuropathy rating scores, monofilament examination is insensitive to changes over 12 months in a mild-to-moderate affected population of this size.

CONCLUSION

Estimation of glycosylated hemoglobin is a simple, rapid, and objective procedure to assess diabetic control. It serves both as a screening test for uncontrolled diabetes and as an indicator of the efficacy of various therapeutic regimens. It also provides a conceptual frame work for the pathogenesis of the long-term complications of diabetes. Its estimation gives a relatively precise reflection of the state of diabetic control as compared to blood glucose estimation. Therefore, it is now possible to estimate more accurately and with greater sensitivity the degree of glucose intolerance; particularly, in cases associated with diabetic complications. It represents an accurate technique to evaluate new ways of controlling blood glucose. Thus, as an integral of diabetic control, glycosylated hemoglobin (HbA1C) estimation is superior to the conventional measures in assessment of control.

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