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RESEARCH ARTICLE

BIOCHEMISTRY

CALCULATED GLYCATED HEMOGLOBIN – MYTH OR REALITY

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ABSTRACT

Diabetes mellitus is a chronic metabolic disorder characterized by rise in blood glucose level and derangement in protein and fat metabolism. The measurement of GHb is one of the well established means of monitoring glycemic control in patients with diabetes mellitus. The aim of this study was to understand the significance of calculated HbA_{1c} by using fasting plasma glucose levels and comparing it with estimated HbA_{1c}. The study population consisted of 100 subjects. We found significance between the estimated and calculated HbA_{1c} levels in the study subjects ($p<0.001$). HbA_{1c} values calculated on the basis of current blood glucose and past HbA_{1c} levels are not actually identical to the HbA_{1c} values present in erythrocytes. However, a good approximation supplied by an appropriate mathematical model would have some advantages over true measurement. Objective of this study was to provide a tool for regular and frequent checking of HbA_{1c} with proper accuracy. As the formula can be used in well controlled diabetes patients only and is not a replacement for estimated HbA_{1c}.

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KEY WORDS

Glycated hemoglobin, diabetes mellitus, fasting plasma glucose

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by rise in blood glucose level and derangement in protein and fat metabolism (1). The worldwide prevalence of diabetes in 2000 was approximately 2.8% and is estimated to grow to 4.4% by 2030. This translates to a projected rise of diabetes from 171 million in 2000 to well over 350 million in 2030. Glycated hemoglobin (*glycosylated hemoglobin*) is a form of hemoglobin used primarily to identify the average plasma glucose concentration over prolonged periods of time. It is formed in a non-enzymatic pathway by hemoglobin's normal exposure to high plasma levels of glucose (2). The measurement of GHb is one of the well established means of monitoring glycemic control in patients with diabetes mellitus as well as in primary diagnosis of diabetes mellitus in certain situations (3).

Studies on chronic complications of diabetes established the role of glycosylated hemoglobin (HbA_{1c}) as a marker of evaluation of long term glycemic control and risk for chronic complications (4). The Diabetes Control and Complication Trial (DCCT) study, has demonstrated that 10% stable reduction in HbA_{1c} determines a 35% risk reduction for retinopathy and a 25- 44% risk reduction for nephropathy (5).

Measurement of HbA_{1c} is recommended for both (a) checking blood sugar control in people who might be pre-diabetic and (b) monitoring blood sugar control in patients with more elevated levels. According to the American Diabetes Association guidelines the glycosylated hemoglobin test can be performed at least two times a year in patients with diabetes who are meeting treatment goals (and who have stable glycemic control) and quarterly in patients with diabetes whose therapy has changed or who are not meeting glycemic goals(6).

The aim of this study was to understand the significance of calculated HbA_{1c} by using fasting plasma glucose levels and comparing it with estimated HbA_{1c}.

MATERIALS AND METHODS

The study population consisted of 100 subjects (age matched subjects) divided in to two groups viz., diabetic patients (type 2 diabetic subjects; n=50) and nondiabetic participants (n=50) admitted in Kasturba Hospital, Manipal. The non-diabetic participants had no history of diabetes and had HbA_{1c} levels in the non-diabetic range (<6.5%) in the assays used for the study. The study was approved by the Institutional Time Bound Research committee. A written informed consent was taken from the subjects.

Exclusion criteria:

- Patients with uncontrolled diabetes mellitus
- Potential participants with known haemoglobinopathies were excluded to avoid any possible interference with the HbA_{1c} assays
- Patients with End stage renal failure

Sample collection:

4ml of fasting venous blood samples were collected in sodium fluoride vacutainers under strict aseptic precautions from all subjects. Age, gender and duration of diabetes were noted. The blood was analyzed for glucose and glycosylated hemoglobin (HbA_{1c}) .

Biochemical estimations:

Plasma glucose was estimated by GOD – POD method (7). Blood was analyzed for HbA_{1c} in auto analyzer using Cobas commercial kit (8). Estimation of glycated hemoglobin was done by calculation (9). **HbA_{1c} = 2.6 +0.03 × Plasma glucose (mg/dl)**, mean blood glucose level of 130 mg/dl(=7.2 mmol/L) would be equivalent to 6.5% HbA_{1c}. Any additional 10 mg/dl (=0.56 mmol/L) translate to an additional 0.3% HbA_{1c}.

Statistical analysis:

Analysis was done by student 't' test using SPSS windows version 10.0 software and results were expressed as mean ±SD. $p<0.05$ was considered statistically significant.

RESULTS

The results of the study are shown in table 1. We found significance between the estimated and calculated HbA_{1c} levels in the study subjects.

Table 1
Glucose and HbA_{1c} levels in study subjects

	Normal subjects (n=50) (mean±SD)	DM Cases (n=50) (mean±SD)
Fasting plasma glucose (mg/dl)	85.38±10.69	143.43±40.35***
Estimated HbA_{1c}%	6.42±0.55	7.43±1.41***
Calculated HbA_{1c}%	5.16±0.32	6.90±1.20***

***= *Very highly significant($p<0.001$)*

DISCUSSION

The relationship between HbA_{1c} and Plasma glucose (PG) is complex. Higher levels of HbA_{1c} are found in subjects with persistently elevated blood sugar, as in diabetes mellitus. A diabetic person with good glucose control has an HbA_{1c} level that is close to or within the reference range. The International Diabetes Federation and American College of Endocrinology (IDFACE) recommend HbA_{1c} values below 6.5%, while American Diabetes Association (ADA) recommends that the HbA_{1c} be below 7.0% for most patients to indicate good glycemic control (10).

Diabetes therapy is mainly about restoring glycemic control to avoid long-term consequences such as impaired vision, kidney failure or angiopathy. Ways to prevent these vascular diseases include dietary measures, oral drugs or insulin substitution. The objective is always to keep blood glucose levels essentially within the normal range. HbA_{1c} values give an indication of mean blood glucose levels over the previous 2 or 3 months and therefore are a suitable parameter to monitor the success of self-performed glycemic control (11).

In the last 20 years improved techniques in laboratory and new electrophoretical, chromatographic and immunological methods

available, gave us a greater reliability on our results. However the use of different methods, the lack of a common calibration concerning the same method and the variability of instrumentation do not make reproducible results yet in different laboratories. The liquid chromatography ionic exchange is now the most reliable methodology.It allows to measure with precision all sub-fractions of HbA_{1c} and anomalous hemoglobins. Its cost is high and it is not available in all the laboratories (12, 13).

In the present study we found a significance between estimated and calculated HbA_{1c} levels in study subjects. The predicted HbA_{1c} values thus obtained were in accordance with measured values. They also matched the results of the HbA_{1c} formula in the elevated range. By contrast, the formula was too "optimistic" in the range of better glycemic control. Individual analysis of two subjects improved the accuracy of values and reflected the bias introduced by different glucometers and individual measurement habits.

HbA_{1c} values calculated on the basis of current blood glucose and past HbA_{1c} levels are not actually identical to the HbA_{1c} values present in erythrocytes. However, a good approximation supplied by an appropriate mathematical model would have some advantages over true measurement. There would be no need for taking additional blood samples as blood glucose values are measured to determine the required insulin doses anyhow. The HbA_{1c} values could be calculated on regular basis and it would also eliminate the cost.

Self-management is becoming increasingly important in diabetes therapy. This is a positive development because greater flexibility will improve quality of life and hence patient acceptance. In addition, personal responsibility contributes to the success of therapy (14, 15).

The main objective of this study was to provide a tool for regular and frequent checking of HbA_{1c} with a proper accuracy. As the formula can be used in well controlled diabetes patients only it is not a replacement for estimated HbA_{1c}.

REFERENCES

1. Aaron & Vinik.Diabetes and macrovascular disease. journal of diabetes and its complications,16:235-245, (2001)
2. Larsen ML, Hørder M, Mogensen EF. Effect of long-term monitoring of glycosylated hemoglobin levels in insulin-dependent diabetes mellitus. N. Engl. J. Med. 323 (15): 1021–5(1990)
3. Diabetic Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long term complications in insulin dependent diabetes mellitus. N. Engl J Med,329:977-986(1993)
4. Lorenza Calisti, Simona Tognetti. Measure of glycosylated hemoglobin. Acta biomed ,76; Suppl. 3: 59-62(2005)
5. Dahl-Jorghensen K, Brichmann-Hanssen O, Hansenn KF, et al. Effect of near normoglycemia for two years on progression of early diabetic retinopathy, nephropathy and neuropathy. The Oslo Study. Br Med J ,293: 1195-9(1986)
6. American Diabetes Association: Standards of medical care in diabetes—2006. Diabetes Care ,29:S4–42(2006)
7. Trinder P. Quantitative determination of glucose using GOD-PAP method. Ann Clin Biochem, 6: 24-27(1969)
8. Gold stein DE, Malone J, Nathan D et al. Tests of glycemia in diabetes, Diabetes care, 18:896-909(1995)
9. Wilhelm T, Anton L, Michaela R. HbA1c values calculated from blood glucose

levels using truncated Fourier series and implementation in standard SQL database language. Methods Inf Med ,47(4): 346-55(2008)

10. "Executive Summary: Standards of medical care in diabetes—2009". Diabetes Care, 32: S6–S12. (2009)

11. DeVries, J.H., et al., Improved glycaemic control in type 1 diabetes patients following participation per se in a clinical trial--mechanisms and implications. Diabetes Metab Res Rev, 19(5): p. 357-62(2003)
12. Schifreen RS, Hickingbotham JM, Bowers GN. Accuracy, precision and stability in measurement of hemoglobin A1c by high performance cation exchange chromatography. Clin Chem ,26: 3-8(1980)
13. Matteucci E, Milioni C, Biasci E, Bertoni C, Boldrini E, Giampiero O. With regard to glycohemoglobin measurement: are we sure that high-performance liquid chromathography currently works in the clinical routine? Acta Diabetologica ,35: 41-7.
14. Kilpatrick, E.S., Problems in the assessment of glycaemic control in diabetes mellitus. Diabet Med, 1997. 14(10): p. 819-31(1998)
15. Kildegaard, J., et al., A study of trained clinicians' blood glucose predictions based on diaries of people with type 1 diabetes. Methods Inf Med, 46(5): p. 553-7(2007)