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Original Research Article

Comparison of the levels of estimated HbA1c with calculated HbA1c - A one year cross sectional study at KLE Dr Prabhakar Kore Hospital, Belagavi

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ABSTRACT

Introduction: Diabetes Mellitus is a group of metabolic disorders characterized by rise in blood glucose level. The measurement of Glycated Hemoglobin has been widely used to monitor glycemic control in patients with diabetes mellitus which is also a best marker for monitoring long term complications. As the prevalence of diabetes is increasing day by day, it is mandatory to give importance for self-management. **Objectives:** The objective of the present study was to compare the levels of estimated HbA1c with calculated HbA1c derived from a simple, cost effective mathematical formula by using fasting plasma glucose.

Materials and Methods: The present study comprised of 66 fasting whole blood samples. All the samples were analyzed for Glycated hemoglobin and glucose, using this blood glucose level HbA1c was calculated by a formula, $HbA_{1c} = 2.6+0.03 \times Blood$ glucose (mg/dl). Both estimated and calculated values of HbA1c were compared by dependent t-test.

Results: It was observed that the mean \pm SD levels of Estimated HbA1c and Calculated HbA1c were 7.11 \pm 1.89 and 6.60 \pm 1.58 respectively. The p value was <0.05.

Conclusion: Therefore the study concluded that, there exists a significant difference between the levels of estimated HbA1c and calculated HbA1c and thus this mathematical formula cannot be used interchangeably with estimated HbA1c.

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1. Introduction

Diabetes Mellitus is a group of metabolic disorders characterized by rise in blood glucose level with disturbed fat, carbohydrate and protein metabolism either because of impaired synthesis and secretion of insulin (Type 1 DM) or impaired sensitivity of tissue to insulin action (Type 2 DM).¹ It is a complex and chronic illness associated with a greater risk of heart diseases, stroke, peripheral neuropathy, renal diseases and blindness which requires continuous medical care with multifactorial risk reduction plan apart from glycemic control.²

Glycated hemoglobin, also known HbA1c is the form of hemoglobin where the glucose molecules are attached Use of HbA1c for the diagnosis of diabetes may identify additional subjects compared to that of fasting glucose level and it is also used in making treatment decisions which has become essential for the assessment of diabetes care.⁵ Increased levels of HbA1c are seen in patients with poorly controlled diabetes than in healthy people and it is also associated with cardiovascular diseases, nephropathy and retinopathy in diabetes mellitus.⁶

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to N-terminal end of beta chain of hemoglobin by nonenzymatic reactions and forms Schiff base which is unstable and undergoes Amadori rearrangement to form HbA1c.³ The measurement of Glycated Hemoglobin has been widely used to monitor glycemic control in patients with diabetes mellitus which is also a best marker for monitoring long term complications as it indicates the mean blood glucose over the previous 2-3 months.⁴

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As the prevalence of diabetes is increasing day by day, it is mandatory to measure the Glycated hemoglobin frequently which is a marker of long term glycemic control and give importance for self-management. There are several improved techniques available to measure the HbA1c but lack of common calibration regarding some methods and the variability of instrumentation ends up in false results. However, the High Performance Liquid Chromatography (HPLC) is the most reliable methodology but its cost is high and it is not obtainable in all the laboratories.⁷

Identification of a tool such as mathematical model or formula can provide a key for regular and frequent checking of HbA1c with proper accuracy, on the similar line many studies have predicted the HbA1c values by using simple mathematical formula or model.

The objective of the present study was to compare the levels of estimated HbA1c with calculated HbA1c derived from a simple, cost effective mathematical formula by using fasting plasma glucose.

2. Materials and Methods

This study comprised of 66 fasting whole blood samples which were obtained from the Dr. Prabhakar Kore Hospital, Belagavi, Karnataka, India. 3ml of fasting blood was collected in an EDTA tube and Glycated Hemoglobin was measured by ION Exchange Resin method.

2.1. Methodology

A hemolysed preparation of the whole blood is mixed continuously for five minutes with a weak binding cation – exchange resin. After the mixing period a filter is used to separate the supernatant containing the glycohemoglobin from the resin.



(HbA1a, HbA1b, HbA1c)

Fig. 1:

The glycohemoglobin percent is determined by measuring the absorbence at 415nm of glycohemoglobin fraction and total hemoglobin fraction. The ratio of the two absorbences gives the percentage Glycohemoglobin.

The remaining sample was centrifuged at 3500 RPM for 10 min to get Clear plasma which was used for the measurement of Glucose by GOD-POD method. Using this blood glucose levels HbA1c was calculated by formula,⁸

HbA1c = $2.6+0.03 \times$ Blood glucose (mg/dl)

Both the levels of estimated and calculated HbA1c were compared. The measurement of Glycated haemoglobin and Glucose of all the samples was done by using ERBA chem 7 semi automated analyzer.

The data obtained was analyzed statistically by computing descriptive statistics, the mean, standard deviation, paired t value and p value were calculated.

3. Results

The mean and standard deviation of calculated HbA1c were found to be lower than that of estimated HbA1c. The paired t value is 3.816 and p value is <0.05, therefore there is a significant difference between the levels of estimated HbA1c and calculated HbA1c.



Fig. 2: Comparison of Estimated HbA1c and calculated HbA1cscores

It was observed that the mean \pm SD levels of Estimated HbA1c and Calculated HbA1c were 7.11 \pm 1.89 and 6.60 \pm 1.58 respectively. These values were compared by t-test where paired t value was found to be 3.81 and the p value was <0.05. Thus, there is a significant difference between estimated HbA1c and calculated HbA1c (Table 1 and Figure 2).

4. Discussion

In the present study, the mean levels of calculated and estimated HbA1c seemed to be similar, but they were actually significantly different (p<0.005). Hence, we observed a significant difference between levels of calculated HbA1c with that of estimated HbA1c.

Our study is in accordance with the study conducted by Bhavana Nayal et al. which showed that HbA1c values calculated on the basis of current blood glucose and past HbA1c levels are not actually identical to the HbA1c values present in erythrocytes and therefore this formula can be used in well controlled diabetes patients only and it is not a replacement for estimated HbA1c.⁷

Another study was conducted by Wilhelm Temsch et al. to calculate HbA1c values based on self-measured blood glucose and past HbA1c levels using a truncated Fourier series which suggested that, predicated HbA1c values are

Variables	Mean	Std.Dv.	Mean Diff.	SD Diff.	Paired t	p -value
Estimated HbA1c	7.11	1.89				-
Calculated HbA1c	6.60	1.58	0.51	1.09	3.8163	0.0003*
*n<0.05						

Table 1: Comparison of Estimated HbA1c and calculated HbA1c by depe	endent t test
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*p<0.05

no exception to the estimated HbA1c and the formula used is liable to create wrong impression and therefore can be used especially in well controlled diabetes patients.⁸

Our study is in contrast with the study done by Dayanand C.D et al. to compare the levels of HbA1c determined by the HPLC method with HbA1c derived from simple calculation using a mathematical model which conformed that, the HbA1c values derived and predicated by formulae were in accordance with measured values by HPLC-BIORAD method and hence a comparative relation exists between measured and calculated with IFCC values of HbA1c in control and type 2 diabetic group.⁹

Another study was conducted by Manjunath Goud B.K et al. to find the relation of calculated HbA1c with fasting plasma glucose and duration of diabetes. According to this study, there is a positive correlation of glucose with calculated HbA1c (r=0.999), suggesting that the formula is applicable and can be used to make regular checkups of HbA1c in diabetic patients with less cost.¹⁰

5. Conclusion

The study concludes that:

- The HbA1c levels calculated from the plasma glucose are not exactly identical with the levels of estimated HbA1c; there exists a significant difference between the levels of estimated HbA1c and calculated HbA1c.
- 2. Therefore, this mathematical formula cannot be used interchangeably with estimated HbA1c.

6. Limitations

The estimation of HbA1c was done by Ion exchange resin method and not by HPLC which is the Gold standard method.

7. Source of Funding

None.

8. Conflict of Interest

None.

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