

Calculated Glycosylated Hemoglobin (HbA1c) Compared with Estimated HbA1c by Nephelometry and Its Correlation to Estimated Average Blood Glucose (eAG)

Shruthi Soundara Rajan¹, Anitha Misquith², Harish Rangareddy³

¹II MBBS Student, Sapthagiri Institute of Medical Sciences & Research Center, Bengaluru-560090

²Assistant Professor, Department of Biochemistry, Sapthagiri Institute of Medical Sciences & Research Center, Bengaluru-560090

³Associate Professor, Department of Biochemistry, Sri Devaraj Urs Medical College, Kolar – 563101

Corresponding Author: Harish Rangareddy

ABSTRACT

Background and Aim: Glycated hemoglobin is a form of hemoglobin used primarily to identify the average plasma glucose concentration over prolonged periods of time. In a developing country like India there are quite a few resource poor settings where HbA1c is not available. In such circumstances, a mathematical tool like calculated HbA1c would have some advantages over true estimation of HbA1c. This study was carried out to explore the feasibility of using such a formula to calculate HbA1c and compare with the plasma fasting and post prandial glucose levels, measured HbA1c; also to correlate with estimated average blood glucose (eAG) assessed from estimated HbA1c.

Materials and Methods: This is a cross sectional study where 50 type 2 diabetes mellitus patients and 50 healthy controls were enrolled. Blood work up done for fasting plasma glucose (FBS), postprandial glucose (PPBS) and HbA1c.

Results: The mean±SD levels of estimated HbA1c are 7.574±1.52% and calculated HbA1c 7.540± 2.29% in diabetic individuals. Estimated HbA1c is 5.629±0.15% and calculated HbA1c 5.302±0.31% in non-diabetic subjects. Though there was no difference between estimated HbA1c and calculated HbA1c by paired 't' test Bland Altman plot analysis with Beta coefficient showed that there was significant bias. Calculated HbA1c correlated positively with estimated average glucose (eAG) only in diabetics but not in controls.

Conclusion: The present study concludes that there is no significant difference observed in

HbA1c measured by Nephelometric method and HbA1c calculated using plasma fasting glucose. However, it may not be suitable for inter changeable usage as evidenced by the bias with beta coefficient.

Keywords: HbA1c protein, Diabetes mellitus, Estimated Average Glucose

INTRODUCTION

Diabetes mellitus is a metabolic disorder monitored by plasma glucose and glycosylated hemoglobin (HbA1c) estimations. Estimated average blood glucose (eAG) refers to an average blood glucose level, expressed in milligrams per deciliter (mg/dl), based on a person's HbA1c. Thus, eAG reflects the average glycemic control over the preceding 3 months^[1]. In clinical biochemistry there is a role for mathematical formulae to calculate some of the analytes of interest and calculated HbA1c is one such mathematical tool which is simple and cost effective^[2]. There is scarcity of data regarding the utility of calculated HbA1c in monitoring glycemic control.

Glycated 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 glucose levels. The measurement of glycated

hemoglobin 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 situation ^{[3], [4]}. HbA1c is not only a useful biomarker of long-term glycemic control but also a good predictor of lipid profile; thus, monitoring of glycemic control using HbA1c could have additional benefits of identifying diabetes patients who are at a greater risk of cardiovascular complications. Thus, a single HbA1c test provides valuable information that can be used for the management of chronic diseases ^[5].

In a developing country like India there are quite a few resource poor settings where HbA1c is not available. In such circumstances, a mathematical tool like calculated HbA1c would have some advantages over true estimation of HbA1c. The HbA1c can be calculated regularly and eliminates the cost. This study was carried out to explore the feasibility of using such a formula to calculate HbA1c and compare with the plasma fasting and post prandial glucose levels, measured HbA1c; also to correlate with estimated average blood glucose (eAG) assessed from estimated HbA1c.

MATERIALS AND METHODS

This is a Cross sectional study carried out from January 2020 to March 2020. The sampling method used was Purposive sampling. Institutional ethics committee of Sapthagiri Institute of Medical Sciences & Research Center, Bengaluru had approved the study.

Fifty patients already diagnosed with type 2 diabetes mellitus (T2DM) visiting to Medicine department and Central Diagnostic Laboratory for follow up investigations at Sapthagiri Institute of Medical Sciences and Research Center, Bengaluru were enrolled for the study. Controls were age and gender matched volunteers who had undergone routine health check up and blood work up done for fasting plasma glucose (FBS), postprandial glucose (PPBS) and HbA1c. Written

informed consent was obtained from all subjects participating in the study.

The exclusion criteria included

- Hemoglobinopathies
- Thyroid disorders
- Hypertension patients on diuretics
- Renal disorders
- Severe anemia
- Pregnancy

Method of sample collection

Universal safety precautions were taken while collecting the blood samples. Sterile disposable needle and vacutainer was used for sample collection. Correct procedure was followed at every step such as site for venepuncture and pressure used to transfer into vacutainer; on the whole the occurrence of hemolysis can be prevented by this.

After obtaining written informed consent about 4ml of venous blood was drawn under aseptic precautions in EDTA lavender top, sodium fluoride containing grey top vacutainers and processed accordingly. Grey top vacutainers were centrifuged at 3000 rpm for 15 minutes and the sample was obtained.

- Fasting and post prandial plasma glucose by Glucose oxidase-Peroxidase method ^[6]
- The EDTA samples were analyzed in Mispa *i3* auto analyzer for HbA1c by nephelometry ^[7]

This method is based on the interaction of antigen and antibody to directly determine the HbA1c in whole blood. Total hemoglobin and HbA1c have the same non specific absorption rate to latex particle. When mouse antihuman HbA1c monoclonal antibodies is added, latex HbA1c – mouse antihuman HbA1c antibody complex is formed. Agglutination occurs when goat anti-mouse IgG polyclonal antibody interacts with the monoclonal antibody. The amount of agglutination is proportional to the amount of HbA1c absorbed onto the surface of latex

particles. The amount of agglutination is measured to calculate HbA1c % from a calibration curve.

ADA recommended reference range:

5.7 – 6.4 % (High risk group)

Above 6.5% (Diabetics) [1]

- Calculated HbA1c using the formula

$$\text{HbA1c} = 2.6 + 0.03 \times \text{FBS (mg/dL)} \quad [4]$$

- Estimated average glucose is measured by using the formula

$$\text{eAG} = 28.7 \times \text{HbA1c} - 46.7 \quad [8]$$

STATISTICAL ANALYSIS

Descriptive data was represented as mean and standard deviation. Comparison between the groups was done by independent 't' test and paired 't' test was applied for comparing estimated HbA1c and calculated HbA1c. Bland Altman plot

analysis with Beta coefficient was performed to check for the bias. Pearson's correlation analysis was used to indicate the association or linear relationship between the calculated HbA1c and eAG. Statistical analysis was carried out using Open EPI info software.

RESULTS

The mean \pm SD levels of estimated HbA1c are 7.574 \pm 1.52% and calculated HbA1c 7.540 \pm 2.29% in diabetic individuals. Estimated HbA1c is 5.629 \pm 0.15% and calculated HbA1c 5.302 \pm 0.31% in non-diabetic subjects. Estimated average plasma glucose is 170.67 \pm 43.63 mg/dL in diabetic cases and 104.5 \pm 4.59 mg/dL in controls. The results of the study are shown in table (1). 'p' value of less than 0.05 was considered statistically significant.

Table (1): Comparison of FBS, PPBS, HBA1c, Calculated HbA1c and estimated Average Glucose (eAG) in cases and controls

Parameters	Cases (n=50)	Controls (n=50)	'p' value
	Mean \pm SD	Mean \pm SD	
FBS (mg/dL)	164.32 \pm 76.58	90.06 \pm 10.41	<0.001
PPBS (mg/dL)	230.9 \pm 86.85	117.46 \pm 18.18	<0.001
Estimated HbA1c (%)	7.57 \pm 1.52	5.26 \pm 0.15	<0.001
Calculated HbA1c (%)	7.54 \pm 2.29	5.3 \pm 0.31	<0.001
eAG (mg/dL)	170.67 \pm 43.62	104.52 \pm 4.58	<0.001

There was no significant difference between estimated HbA1c and calculated HbA1c when checked with paired t test

suggesting that the calculated HbA1c correlated well with the estimated HbA1c as shown in table (2) and table (3).

Table (2): Comparison of estimated HbA1c and calculated HbA1c among diabetics by paired 't' test

	Mean	Std. Deviation	t	p value
Estimated HbA1c %	7.57	1.52	0.161	0.873
Calculated HbA1c %	7.54	2.29		

Table (3): Comparison of estimated HbA1c and calculated HbA1c among non-diabetics by paired 't' test

	Mean	Std. Deviation	t	p value
Estimated HbA1c %	5.26	0.15	-0.649	0.519
Calculated HbA1c %	5.3	0.31		

The calculated HbA1c showed a positive correlation with estimated average glucose in diabetics Figure (1) which was significant. However, in non-diabetics the association was not significant Figure (2).

Bland Altman plot was done with beta coefficient to check if there was any proportional bias and it showed a statistically significant negative bias as shown in Figure (3) for diabetics and Figure (4) for non-diabetics.

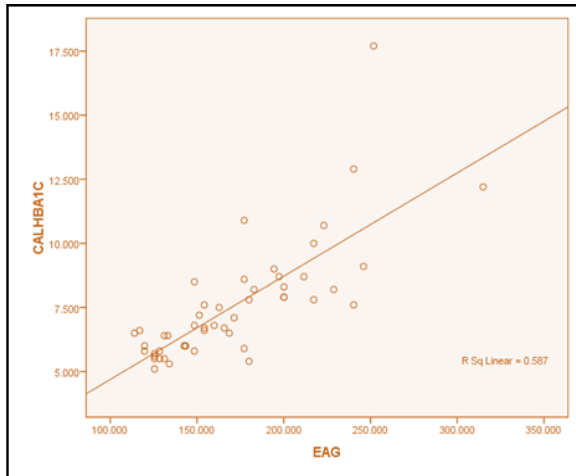


Figure (1) Pearson's correlation of calculated HbA1c with eAG in diabetics

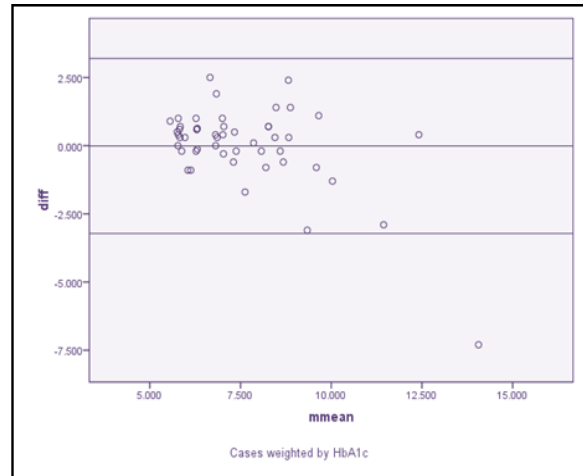


Figure (3) Bland Altman plot for agreement between estimated HbA1c and calculated HbA1c in diabetics

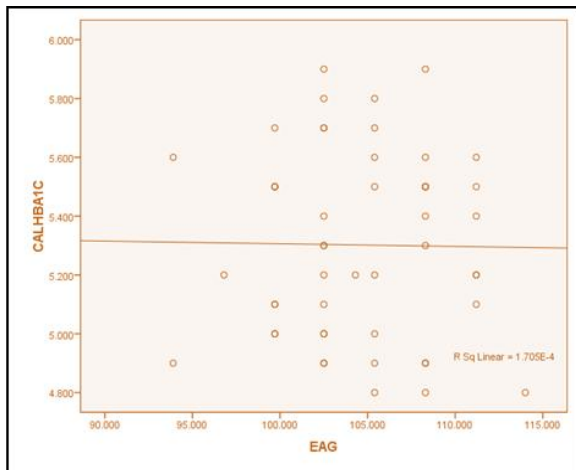


Figure (2) Pearson's correlation of calculated HbA1c with eAG in non-diabetics

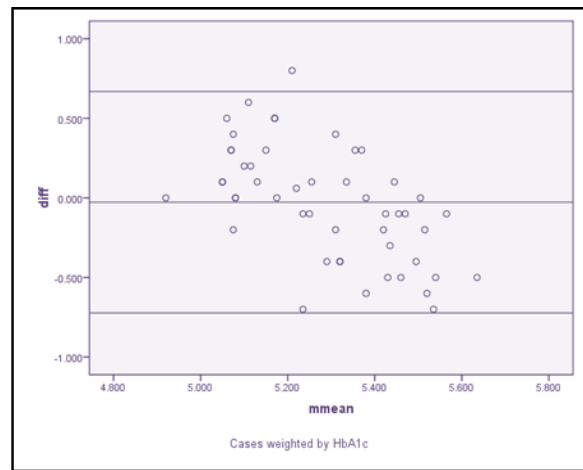


Figure (4) Bland Altman plot for agreement between estimated HbA1c and calculated HbA1c in non-diabetics

Beta Coefficients						
Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	3.760	0.293		12.821	<0.001
	mmean	-0.479	0.036	-0.564	-13.246	<0.001
p<0.001						

Beta Coefficients						
Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	6.196	0.545		11.371	<0.001
	mmean	-1.177	0.103	-0.577	-11.428	<0.001
p<0.001						

DISCUSSION

Hyperglycemia is strongly and independently associated with the complications of type 2 diabetes, including diabetes-related and all-cause mortality, even after adjusting for other metabolic abnormalities often present in this population, such as hypertension and

hyperlipidemia^[9]. The American Diabetes Association (ADA) has been actively concerned in the development and distribution of diabetes care clinical practice recommendations and its Standards of Medical Care is viewed as an important resource for health care professionals who care for people with diabetes^[10]. Long term

near normoglycemia may prevent the progression of early stages of late diabetic complications^[11].

The diabetes complications are equally associated with the both types of DM. Defects in insulin metabolism and dysfunction in carbohydrate, lipid and protein metabolism leads to high blood levels of glucose which results in long-term complications. Diabetic complications include hypertension, retinopathy, end-stage renal disease, neuropathy, peripheral vascular disease, electrolyte imbalance, immune suppression, erectile dysfunction, and complications of pregnancy. Any condition leading to deterioration in glycemic control necessitates more frequent monitoring of blood glucose. The diabetes control and complication trial study has demonstrated that 10% stable reduction in HbA1c determines 35% risk reduction for retinopathy and a 25 to 44% for nephropathy^{[12], [13], [14]}.

Different epidemiologic studies and clinical trials have explored the relationship between HbA1c and the average blood glucose. In particular, the HbA1c-derived average glucose examined the link between HbA1c and the average glucose assessed as completely as possible with combinations of continuous glucose monitoring and frequent finger stick capillary glucose testing. The HbA1c -derived average glucose examined the link between the glycated haemoglobin and the estimated average glucose (eAG), and provided a linear relation between them. During the last two decades, different mathematical models were proposed to deal with the relationship between HbA1c and the average glucose^{[15], [16]}.

In this study there was no significant difference between the estimated HbA1c and calculated HbA1c but however there was a significant bias when the Bland Altman plot was made in both diabetics and non-diabetics. The bias in diabetics was probably due to one individual with uncontrolled diabetes mellitus and hyperglycemia. This implies that in diabetics with good glycemic control the

formula may be helpful. This finding is in accordance with the study by Temsch W et al who calculated HbA1c based on self measured glucose and past HbA1c values using truncated Fourier series; observed that the HbA1c calculated using mathematical formula is liable to wrong interpretation and can be used in diabetics with good glycemic control^[4].

The finding of our study is in accordance with the studies done by Nayal B et al, who observed that the erythrocyte HbA1c level was not identical with the calculated HbA1c levels from current blood glucose levels. The formula can be used in well controlled diabetes patients only and is not a replacement for estimated HbA1c^[3]. Desai NG et al, observed that there was a significant difference between the estimated HbA1c using ion exchange resin and calculated HbA1c by dependent 't' test^[17]. In study done by Dayanand et al, observed that calculated HbA1c values conformed to estimated HbA1c values by HPLC method which is in contrast to our study where the method used is nephelometry^[2].

In this study a mathematical formulae been used to calculate the HbA1c by using the measured fasting plasma glucose by Glucose oxidase- Peroxidase method. This will help the patient to minimize the additional cost to estimate the HbA1c level than the chemical method in resource poor setting. The relationship between HbA1c and plasma glucose is complex. Thereby measuring the accurate plasma glucose with strict monitoring of quality control plays an important role in this. To avoid pre analytical and analytical errors measuring the glucose level by Glucometer should be avoided. Higher levels of HbA1c are found in subjects with persistently elevated blood sugar, as in uncontrolled diabetes mellitus. A diabetic person with good glucose control has an HbA1c level that is close to or within the reference range and in such individuals the mathematical formula may provide identical values.

Limitations of the Study

The method used in this study for the estimation of HbA1c is nephelometry and the gold standard method is HPLC.

CONCLUSIONS

The present study concludes that there is no significant difference observed in HbA1c measured by Nephelometric method and HbA1c calculated using plasma fasting glucose. However, it may not be suitable for inter changeable usage as evidenced by the bias with beta coefficient. There was a significant positive relationship between calculated HbA1c and eAG in diabetic individuals.

The method used for HbA1c estimation is nephelometry and it has to be compared with HPLC method if there is a good correlation between estimated HbA1c and calculated HbA1c in a larger population.

ACKNOWLEDGEMENT

This study was funded by Rajiv Gandhi University of Health Sciences, Bengaluru as part of the Student Research Grants 2019.

REFERENCES

1. Care D. 6. Glycemic targets: standards of medical care in diabetes—2019. *Diabetes Care*. 2019 Jan 1;42(Supplement 1):S61-70.
2. Dayanand CD, Vegi PK, Kutty AVM. Comparison of calculated with measured by high pressure liquid chromatography method. *Int J Sci Innov Discov*. 2012;2(6):573–575.
3. Nayal B, Manjunatha Goud BK, Raghuvver CV, Sarsina Devi O, Devaki RN, Shivashanker S et al. Calculated glycated hemoglobin - Myth or reality. *International Journal of Pharma and Bio Sciences*. 2011 Dec 1;2(1):492-496.
4. Temsch W, Luger A, Riedl M. HbA1c values calculated from blood glucose levels using truncated fourier series and implementation in standard SQL database language. *Methods of information in medicine*. 2008 Apr; 47(4):346-55.
5. Lippi G, Targher G. Glycated hemoglobin (HbA1c): old dogmas, a new perspective?

- Clinical Chemistry and Laboratory Medicine (CCLM). 2010 May 1;48(5):609-14.
6. Trinder P. Determination of blood glucose using an oxidase-peroxidase system with a non-carcinogenic chromogen. *Journal of clinical pathology*. 1969 Mar 1;22(2):158-61.
7. Prathima MB, Reshma S, Shetty P, Janice DS, Kalal BS, Madan GR. Estimation of Glycated Haemoglobin by Nephelometry, Ion Exchange Resin and High Performance Liquid Chromatography: A Cross-sectional Study. *Journal of Clinical & Diagnostic Research*. 2020 Sep 1; 14(9).
8. Bozkaya G, Ozgu E, Karaca B. The association between estimated average glucose levels and fasting plasma glucose levels. *Clinics (Sao Paulo)*. 2010; 65(11):1077-80. doi: 10.1590/s1807-59322010001100003. PMID: 21243275; PMCID: PMC2999698.
9. Sherwani SI, Khan HA, Ekhzaimy A, Masood A, Sakharkar MK. Significance of HbA1c test in diagnosis and prognosis of diabetic patients. *Biomarker insights*. 2016 Jan;11:BMI-S38440.
10. Care D. Standards of Medical Care in Diabetes 2020. *Diabetes Care*. 2020 Jan 1;43:S1-224.
11. Beck-Nielsen H, Olesen T, Mogensen CE, Richelsen B, Olsen HW, Ehlers N, Charles P, Sørensen NS. Effect of near normoglycemia for 5 years on progression of early diabetic retinopathy and renal involvement. *Diabetes research (Edinburgh, Scotland)*. 1990 Dec;15(4):185.
12. Adapa D, Sarangi TK. A review on diabetes mellitus: complications, management and treatment modalities. *J. Med. Health. Sci*. 2015; 4(3).
13. Davies MJ, D'Alessio DA, Fradkin J, Kernan WN, Mathieu C, Mingrone G, Rossing P, Tsapas A, Wexler DJ, Buse JB. Management of hyperglycaemia in type 2 diabetes, 2018. A consensus report by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). *Diabetologia*. 2018 Dec 1;61(12):2461-98.
14. Nathan DM, DCCT/Edic Research Group. The diabetes control and complications trial/epidemiology of diabetes interventions and complications study at 30 years:

- overview. *Diabetes care.* 2014 Jan 1;37(1): 9-16.
15. Boutayeb W, Boutayeb A, Lamlili M, El Mostafa SB, Zitouni N. Simulation of a computed HbA 1c using a weighted average glucose. *SpringerPlus.* 2016 Dec 1;5(1):226.
16. Hoshino J, Molnar MZ, Yamagata K, Ubara Y, Takaichi K, Kovesdy CP, Kalantar-Zadeh K. Developing an HbA1c-based equation to estimate blood glucose in maintenance hemodialysis patients. *Diabetes Care.* 2013 Apr 1;36(4):922-7.
17. Desai NG, Bubanale VS. Comparison of the levels of estimated HbA1c with calculated HbA1c-A one year cross sectional study at KLE Dr Prabhakar Kore Hospital, Belagavi. *International Journal of Clinical Biochemistry and Research.* 2020 Jul 24; 7(2): 251-3.

How to cite this article: Rajan SS, Misquith A, Rangareddy H. Calculated glycosylated hemoglobin (HbA1c) compared with estimated hba1c by nephelometry and its correlation to estimated average blood glucose (eAG). *Gal Int J Health Sci Res.* 2020; 5(4): 65-71.
