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COMPARISON OF CALCULATED HBA$_{1c}$ WITH MEASURED HBA$_{1c}$ BY HIGH PRESSURE LIQUID CHROMATOGRAPHY METHOD

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ABSTRACT

AIM: Diabetes mellitus is a metabolic disorder assessed by measurement of plasma glucose and Glycated hemoglobin (HbA$_{1c}$) that denotes the preceding mean blood glucose value. Measurement of HbA$_{1c}$ along with glucose level found beneficial to know the monitoring status that prevents the onset of associated complications. The present study is taken up to compare HbA$_{1c}$ value determined by the HPLC method with HbA$_{1c}$ derived from a simple cost effective calculation using a mathematical tool. This study further presents the values obtained in terms of IFCC units and compared with Standards.

MATERIALS AND METHODS: 60 Diabetic subjects, 60 healthy controls attending to R. L. Jalapa hospital, Kolar were recruited in the study. HbA$_{1c}$ Measured by HPLC method calculated HbA$_{1c}$ using Plasma glucose and converted to the IFCC Norms.

RESULTS: The Mean ± SD levels of measured HbA$_{1c}$ 8.9 ± 2.02 %, calculated HbA$_{1c}$ 8.1 ± 3.07% fasting plasma glucose 182.58 ± 10.2 mg/dl in diabetic and measured HbA$_{1c}$ 5.8 ±0.65 %, calculated HbA$_{1c}$ 4.8 ± 0.35 %, and Fasting plasma glucose 79.12 ± 11.7 mg/dl in control. The IFCC values in Diabetic and Control group for measured and estimated HbA$_{1c}$ are 70mmol/mol (8.9%) compared to standard IFCC Value 64mmol/mol, 37.4mmol/mol (5.8%) compared to standard 31mmol/mol respectively. Similarly in diabetics, for calculated HbA$_{1c}$ 85 mmol/mol (9.8%) compared to standard 86mmol/mol, in control group for calculated HbA$_{1c}$ 61 mmol/mol (7%) compared to standard 53 mmol/mol.

CONCLUSION: This study concludes, a comparative relation observed between measured and calculated with IFCC values of HbA$_{1c}$ in control and type2 diabetic group.

Keywords: Glycated Hemoglobin, IFCC, Diabetes, HPLC.

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized with hyperglycemia and impairment in secretion or action of endogenous insulin. Although, the etiology of this disease is not well defined, viral infection, autoimmune disease, and environmental factors have been implicated [1-5]. While exogenous insulin and other medications can control many aspects of diabetes associated with vascular system, kidney, retina, lens, peripheral nerves and skin. If untreated, these become extremely costly in terms of longevity and quality of life.

The most prevalent form of diabetes mellitus is type 2 diabetes mellitus (type 2 DM), that onset in adult life due to decline insulin secretion accelerated by various genetic factors [6, 7]. The underlying metabolic cause of this disease is either impairment in insulin-mediated glucose disposal or defective secretion of insulin by pancreatic β-cells or both. Insulin resistance can also develop from obesity, physical inactivity and genetic susceptibility [8, 9]. Insulin resistance typically accompanied by cardiovascular risk factors such as dyslipidemia, hypertension, and prothrombotic factors [10, 11].

The long exposure of the hemoglobin to glycemic status particularly to glucose results the formation of Glycated hemoglobin through aldemine arrangements via Schiff's base formation. Thus the levels of the glucose value results the formation of proportionate rise in HbA_{1c}. Therefore, HbA_{1c} is increased in diabetic subjects with critical hyperglycemia compared to basal value in healthy control group. The determination of low density lipoprotein by Friedwald formulae [12], anion gap[13] osmolality[14] estimated GFR[15] are being practiced in clinical chemistry using calculation by formula. In the similar line of work, in this study, an attempt has been made to find out the relationship and reproducibility of calculated Glycated hemoglobin using fasting plasma glucose value and with the measured true Glycated hemoglobin with HPLC method. However, similar study has been reported by measuring HbA_{1c} using adsorption chromatographic method [16]. A newer aspect of this study is to compare the Glycated hemoglobin measured by HPLC BIORAD USA with Glycated hemoglobin obtained by formulae and converting these values in mmol/mol in accordance of IFCC norms using formula which is being mandatory to represent Glycated hemoglobin [17].

The treatment regimen of diabetes mellitus involves the monitoring of glycemic control [18, 19, 20] to avoid long term associated complications. The main purpose of the treatment is to keep the blood glucose value in normal range. HbA_{1c} is good marker of glycemic status that predicts preceding the mean blood glucose levels for past four months. Therefore it is suitable parameter for glycemic status and established biochemical parameter in treatment of diabetes from the past few decades [21, 22, and 23]. HbA_{1c} calculated by appropriate formulae have some advantage over true measurement such as noninvasive, avoids collection of additional blood sample, values can be calculated at fingertip, more convenient than measurement of HbA_{1c} by kit method, eliminate the cost incurred and decreased the hassle of kit procurement. The objective from our study is to provide an assessment benefit of calculated HbA_{1c} at push off button over measured HbA_{1c} in control and type 2 DM patients and for risk assessment.

MATERIALS AND METHODS

Sixty patients with type 2 DM and sixty normal subjects visited to R. L Jalapa Hospital, Kolar, Karnatakawere taken into the study after obtaining informed consent form and ethical clearance from the institution. Normal subjects were volunteers aged between 45-70 years. Alcoholism, smoking, hypertension, diarrhea, use of diuretics and renal disorders were excluded in both the controls and cases to avoid interferences.

Procedure

Three ml of fasting venous blood was collected in to EDTA tube, aliquated for measurement of glycosylated hemoglobin (HbA_{1c}) by Bio-Rad HPLC method for the mean glucose level. The remaining sample centrifuged at 3500 g at 4°C to obtain the clear plasma which was used for quantification of glucose by Glucose oxidase per oxidase method using the dry chemistry analyzer vitros 250 Johnson and Johnson. HbA_{1c} Measured by HPLC Bio-Rad method is compared with calculated HbA_{1c} using Plasma glucose by Wilhelm T& coworkers [24] using formula 2.6+0.03 X plasma glucose (mg/dl). These results were converted in to the International Federation of Clinical Chemistry and Laboratory medicine norms - IFCC using formula [IFCC- HbA_{1c} (mmol/mol) = Diabetes control and complication trail (DCCT) HbA_{1c} (%) – 2.15) X 10.929] which is being mandatory to represent Glycated hemoglobin value [17] and were compared with the IFCC standard values. The results were analyzed using windows 7 SPSS Excel spread sheet.

RESULTS

The Mean ± SD levels of measured HbA_{1c} 8.9 ± 2.02 %, calculated HbA_{1c} 8.1 ± 3.07% fasting plasma glucose 182.58 ± 10.2 mg/dl in type 2 DM and measured HbA_{1c} 5.8±0.65 %, calculated HbA_{1c} 4.8 ± 0.35 %, and Fasting plasma glucose 79.12 ± 11.7 mg/dl in control group as shown in table 1and figure 1.

Table 1: Comparison of Plasma glucose, measured and calculated HbA_{1c} between control and Type II diabetes

Groups	plasma glucose	Measured HbA_{1c} %	Calculated HbA_{1c} %
controls	79.12 ± 11.74	5.79 ± 0.66	4.97 ±0.35
Type 2 DM	182.58 ± 102.42	8.86 ± 2.24	8.08 ± 3.07
p value	<0.001	<0.001	<0.001

Table 2: Comparison of HbA_{1c} presentation in % as well as IFCC mmol/mol units between control and Type II diabetes

Groups	Measured HbA_{1c} %	IFCC values mmol/mol
Controls	5.79 ± 0.66	37.4 ± 6.7
Type 2 DM	8.86 ± 2.24	70.04 ± 20.8

Table 3: Comparison of calculated HbA_{1c} presentation in % as well as in terms of IFCC mmol/mol units between control and Type II diabetes

Groups	Calculated HbA_{1c} %	IFCC values mmol/mol
controls	4.97 ± 0.35	30.86 ± 3.8
Type 2 DM	8.08 ± 3.07	62.46 ± 26.9

Table 4: Comparison of IFCC units for HbA_{1c} in % against standard IFCC units between measured and calculated HbA_{1c} in control and Type II diabetes

	Measured HbA1c		Calculated HbA1c	
Groups	Units in % on conversion to IFCC value	IFCC Standard value(mmol/mol)	Units in % on conversion to IFCC value	IFCC Standard value (mmol/mol)
controls	37.4 ± 6.7	35.34	30.86 ± 3.8	30.38
Type 2 DM	70.04 ± 20.8	73.8	62.46 ± 26.9	64.64

The IFCC values in Diabetic and Control group for measured and estimated HbA_{1c} are 70mmol/mol (8.9%) as shown in table 2 and figure 2 in comparison to standard IFCC Value 64mmol/mol, 37.4mmol/mol (5.8%) compared to standard 31mmol/mol respectively in table 3 as well as figure 3. Similarly in diabetic group for calculated HbA1c 85 mmol/mol (9.8%)

compared to standard IFCC 86mmol/mol, in control group for calculated HbA1c 61 mmol/mol (7%) compared to standard 53 mmol/mol as shown in table 4 and figure 3.

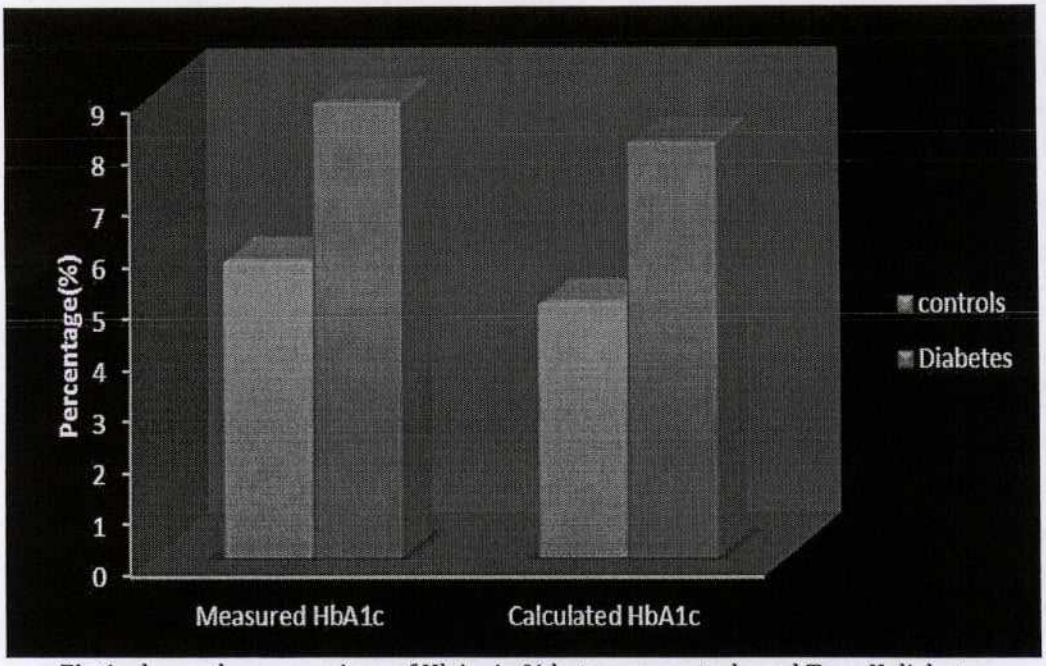

Fig 1: shows the comparison of HbA_{1c} in % between controls and Type II diabetes

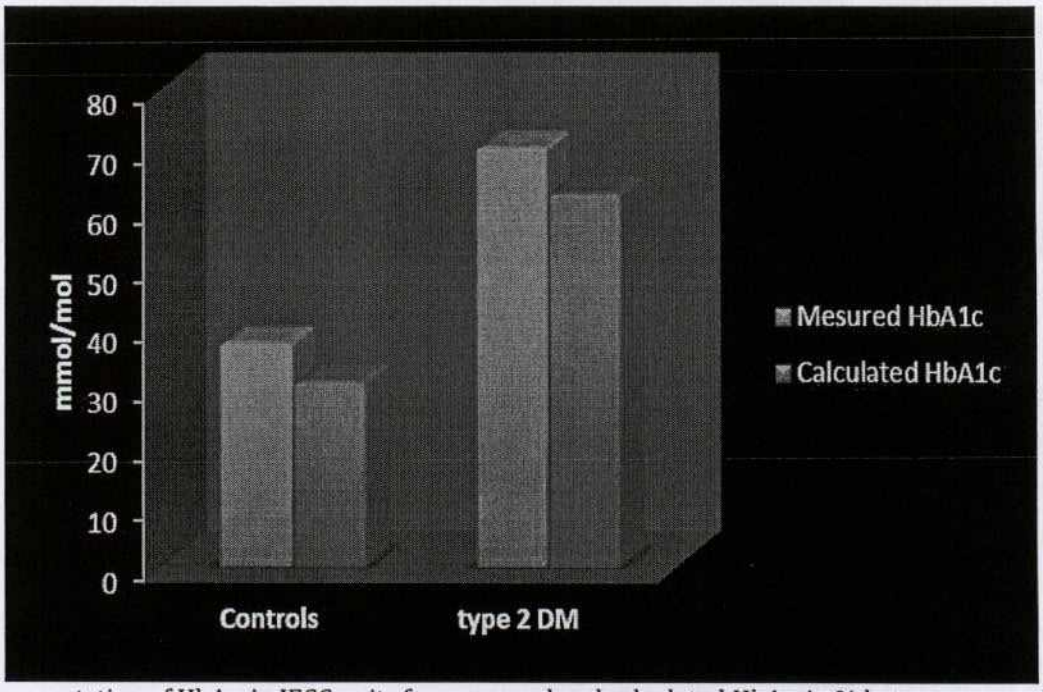

Fig 2: shows presentation of HbA_{1c} in IFCC units for measured and calculated HbA_{1c} in % between controls and Type II diabetes

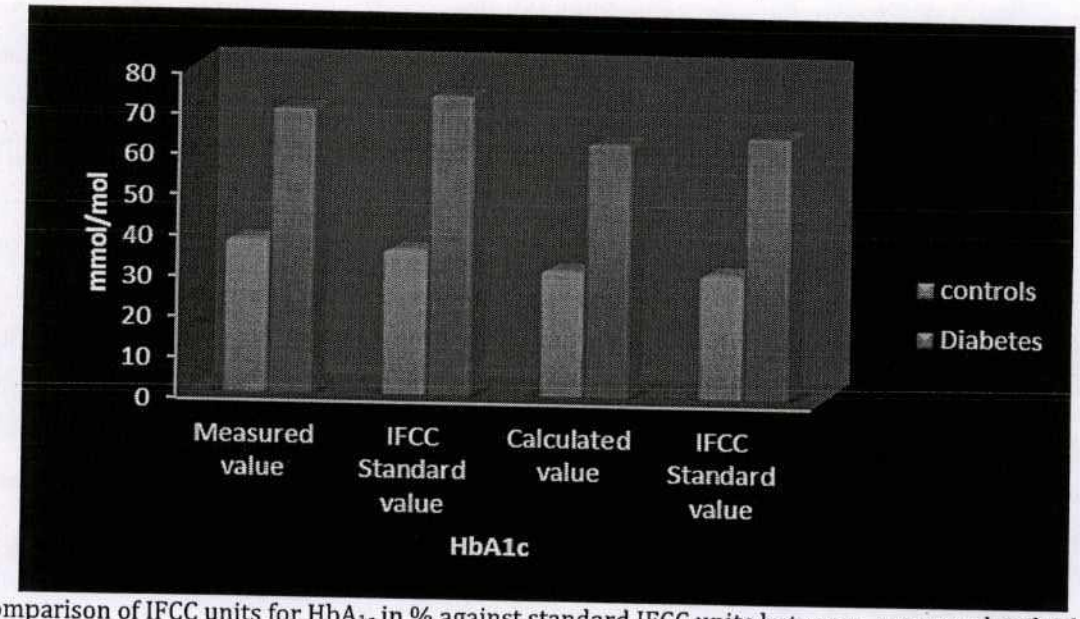

Fig 3: shows Comparison of IFCC units for HbA_{1c} in % against standard IFCC units between measured and calculated HbA_{1c} in controls and Type II diabetes

DISCUSSION/CONCLUSION

In this study a mathematical model is used to calculate HbA_{1c} by utilizing measured fasting plasma glucose level enable to monitor intermittent HbA_{1c} levels between scheduled checkups of the patients on diabetic therapy, this prevents any additional cost or effort involved in HbA_{1c} measurement. The formulae adapted in this study found apparently simple, faster, cost effective and reliable than the chemical analysis. This simple procedure might help to gauge the HbA_{1c} value with mere true fasting glucose level. Even though, the results obtained were more relevantly comparable to measured HbA_{1c}, however the rate of usage of this method is not predominantly practiced. Thereby the measurement of HbA_{1c} by chemical analysis stands mandatory.

The study concludes that HbA_{1c} value derived and predicted by formulae method were in accordance with measured values by HPLC- BIORAD method. The formula absolutely depends on the need of true and accurate plasma glucose value under strict monitoring of quality control. But generally in practice it creates an element of bias in individuals when their glucose value analyzed by glucometer of various types, pre analytical and analytical errors occurred in individual laboratory methods or individual measurement habits practiced.

In this study, the value of HbA_{1c} obtained by measurement and calculation from control and type 2 DM were converted into IFCC values using the formulae in a similar way of Kolb H and co-workers [25]. Accordingly, in both methods HbA_{1c} values were found correlated with the standards of IFCC HbA_{1c} values.

This agreed calculation formulae adapted in this study area to convert Values obtained in percentage according to Diabetes control and complication trail (DCCT) units is appropriate to convert to International federation of clinical chemistry (IFCC) units in mmol/mol as it is mandatory to represent the HbA_{1c} value.

The present study concludes that there is a comparative relation observed in HbA_{1c} measured by HPLC method and HbA_{1c} calculated using plasma fasting glucose and also their conversion to IFCC units in comparison to standard IFCC values in controls and type 2 Diabetic subjects. However, the similar observation needs to be explored in large number of subjects.

REFERENCES

1. Kataoka S, Satoh J, Fujiya H, Toyota T, Suzuki R, Itoh K, Kumagai K. Immunologic aspects of the nonobese diabetic (NOD) mouse. Abnormalities of cellular immunity. Diabetes 1983; 32(3):247–253.
2. Like AA, Rossini AA, Guberski DL, AppelMC,Williams RM. Spontaneous diabetes mellitus: Reversal and prevention in the BB/W rat with antiserum to rat lymphocytes. Science 1979; 206(4425):1421–1423.
3. Paik SG, Blue ML, Fleischer N, Shin S. Diabetes susceptibility of BALB/cBOM mice treated with streptozotocin. Inhibition by lethal irradiation and restoration by splenic lymphocytes. Diabetes 1982; 31(9):808–815.
4. Sandler S, Andersson AK, Barbu A, Hellerstrom C, Holstad M, Karlsson E, Sandberg JO, Strandell E, Saldeen J, Sternesjo J, Tillmar L, Eizirik DL, Flodstrom M, Welsh N. Novel experimental strategies to prevent the development of type 1 diabetes mellitus. Ups J Med Sci 2000; 105(2):17–34.
5. Shewade Y, Tirth S, Bhonde RR. Pancreatic islet-cell viability, functionality and oxidative status remain unaffected at pharmacological concentrations of commonly used antibiotics in vitro. J Biosci 2001; 26(3):349–355.
6. Pimenta W, Korytkowski M, Mitrakou A, Jenssen T, Yki-Jarvinen H, Evron W, Dailey G, Gerich J. Pancreatic beta-cell dysfunction as the primary genetic lesion in NIDDM: evidence from studies in normal glucose-tolerant individuals with a first-degree NIDDM relative. JAMA. 1995; 273:1855–1861.
7. Humphriss DB, Stewart MW, Berrish TS, Barriocanal LA, Trajano LR, Ashworth LA, Brown MD, Miller M, Avery PJ, Alberti KG, Walker M. Multiple metabolic abnormalities in normal glucose tolerant relatives of NIDDM families. Diabetologia. 1997; 40:1185–1190.
8. Gerick JE. The genetic basis of type 2 diabetes mellitus: impaired insulinsecretion versus impaired insulin sensitivity. Endocr Rev. 1998; 19:491–503.
9. Chisholm DJ, Campbell LV, Kraegen EW. Pathogenesis of the insulinresistance syndrome (syndrome X). ClinExpPharmacol Physiol. 1997; 24:782–784.
10. Hopkins PN, Hunt SC, Wu LL, Williams GH, Williams RR. Hypertension, dyslipidemia, and insulin resistance: links in a chain or spokes on a wheelCurrOpinLipidol. 1996; 7:241–253.
11. Gray RS, Fabsitz RR, Cowan LD, Lee ET, Howard BV, Savage PJ. Risk factor clustering in the insulin resistance syndrome: the Strong Heart Study. Am J Epidemiol. 1998; 148:869–878.
12. Friedewald WT, Levy R I, Fredrickson DS. Estimation of the concentration of low density cholesterol in plasma without using preparative centrifuge. Clin Chem. 1972:18:499-502
13. Jianxin Lu, Gordon A. Zello, Edward Randell et al Closing the anion gap: Contribution of D-lactate to diabetic ketoacidosis ClinicaChimicaActa 412 (2011) 286–29.
14. Hung WL, Hsieh PF, Lee YC, Chang MH. Occipital lobe seizures related to marked elevation of hemoglobin A1C: report of two cases.Seizure. 2010 Jul; 19(6):359-62.
15. Hiroki Yokoyama, SakikoKanno, Suguho Takahashi et al Risks for glomerular filtration rate decline in association withprogression of albuminuria in type 2diabetes. Nephrol Dial Transplant (2011) 0: 1–7.
16. ManjunathaGoud B. K, Raghuveer C.V, et al. Calculated glycated hemoglobin – myth or reality International Journal of Pharma and Bio sciences.,Jan-march 2011vol 2/ I-1.

17. D.orazio et al ClinChem Lab Med Approved IFCC recommendation on reporting results for blood glucose: international federation of and labotarory medicine scientific division working group on selective electrodes and point of care testing (IFCC-SD-WG-SEPOCT). 2006:44(12): 1486-1490.
18. 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 MetabRes Rev, 2003. 19(5): p. 357-62).
19. Nomura, D.M., Importance of using and understanding self-monitoring of blood glucose (SMBG) data in assessing ambient and long-term glycaemic control. J Indian Med Assoc, 2002.,100(7): p. 448, 450-1.
20. Rodgers, J. and R. Walker, Glycaemic control in type 2 diabetes. Nurs Times, 2002. 98(19): p. 56-7.
21. Jeffcoate, S.L., Diabetes control and complications: the role of glycatedhaemoglobin, 25 years on diabetes. Diabet Med, 2004.21(7): p. 657-65.
22. Schernthaner, G., et al., [The clinical importance of glycohaemoglobin (HbA1c) Wien Klin WochenschrSuppl, 1980. 115: p. 1-11.
23. Bunn, H.F., K.H. Gabbay, and P.M. Gallop, The glycosylation of hemoglobin: relevance to diabetes mellitus. Science, 1978. 200(4337): p. 21-7.
24. Wilhelm T, Anton L, Michaela R. HbA1c values calculated from blood levels using truncated Fourier series andimplementation in standard SQL databaselanguage. Methods InfMed ,47(4): 346-55(2008).
25. Kolb H Mandrup -poulsen T An immune origin of Type 2 Diabetes Diabetologia 2005 48: 1038-1050.