Glycated Haemoglobin for the Diagnosis of Diabetes Mellitus and to Determine Estimated Average Glucose (EAG) Concentration

Amrita Karmakar [1], Jayati Roy Choudhury[1], Barnita Guha[1],
Jayanta Kumar Rout [2]

[1], MD(Biochemistry) (The West Bengal University of Health Sciences);2-Assistant Professor(R.G.Kar Medical College)

Abstract: Glycated haemoglobin (HbA1c) is widely used to determine levels of chronic glycaemia, to judge the adequacy of diabetes treatment and to adjust therapy. In this study we want to find out that HbA1c >/=6.5% is corroborative with the existing diagnostic criteria (FPG >126mg/dl) of diabetes mellitus (DM) or not and to find out the relationship, if any, between HbA1c and fasting plasma glucose concentration.

Material & Method: 92 diabetic and 107 non-diabeticpatients are included in this present study. Fasting plasma glucose (FPG) was measured by glucose oxidase-peroxidase method and glycated haemoglobin by ion exchange resin method.

Result: It can be said that if >6.5% of $HbA1_C$ is used for the diagnosis the sensitivity and specificity would be about 92% and 91% respectively. From regression analysis, average estimated plasma glucose may be predicted from HbA1C level. **Average glucose (eAG) concentration (mg/dl) = 28.184*$HbA1_C$ − 45.055** Therefore, from this study, it can be concluded that HbA1c >/=6.5% is corroborative with the existing diagnostic criteria (FPG >126mg/dl) of diabetes mellitus. Average blood glucose can be estimatedfrom HbA1c. It can be useful as simple screening tests for type 2 diabetes mellitus (Type 2DM) so patients can be identified earlier and more efficiently.

Keyword-estimated average glucose, glycated haemoglobin

I.   Introduction

Diabetes mellitus (DM) comprises a group of common metabolic disorders that share the phenotype of hyperglycemia(1). The metabolic dysregulation associated with DM causes secondary pathophysiologic changes in multiple organ systems that impose a tremendous burden on the individual with DM and on the health care system(2). With an increasing incidence worldwide, DM will be a leading cause of morbidity and mortality for the foreseeable future.DM is classified on the basis of the pathogenic process into type1 DM & type 2 DM.

According to international Diabetes Federation 371 million people suffer from DM in the world, 61.3 million people in India(3). Major form of this epidemic is type 2 DM(4).The National Urban Survey conducted across the metropolitan cities of India reported that prevalence of diabetes mellitus 11.7 per cent in Kolkata (Eastern India) (5). In adult urban Indian populations varies from a low of 5.4% in a northern state to a high of 12.3–15.5% in Chennai, South India, and 12.3–16.8% in Jaipur, Central India (6)..

Criteria for the Diagnosis of Diabetes Mellitus(1, 7)

• Symptoms of diabetes plus random blood glucose concentration >200 mg/dL[a] *or*
• Fasting plasma glucose >126 mg/dL[b] *or*
• Two-hour plasma glucose >200 mg/dL) during an oralglucose tolerance test[c]

a- Random is defined as without regard to time since the last meal.
b- Fasting is defined as no caloric intake for at least 8 h.
c- The test should be performed using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water;

There is a need to simplify screening tests for type 2 DM, so patients can be identified earlier and more efficiently. An International Expert Committee recently recommended glycated haemoglobin (HbA1c) as a better method than measurement of glucose due to ease of use: not necessarily in the fasting state. The HbA1c result reflects longer term glycaemia and is less affected by recent physical/emotional stress (8).Based on its lower day-to-day variability HbA1c considered as 'gold standard' for monitoring metabolic control in diabetes mellitus(9). HbA1c is widely used to determine levels of chronic glycaemia, to judge the adequacy of diabetes treatment and to adjust therapy. (10). However there is considerable disparity in the age of subjects and many studies reported only crude prevalence rate (11) Therefore, there is an urgent need for a large well-planned

national study, which could provide reliable nationwide data, not only on prevalence of diabetes, but also on pre-diabetes, and the complications of diabetes in India. A study of this nature will have enormous public health impact and help policy makers to take action against diabetes in India.

In this study we want to determine the relationship between HbA1c and estimated average glucose concentration (eAG) and concluded that for most patients withDM, HbA1c can be expressed (with reasonable precision) as an eAG in the same units as our monitoring.

II. Material And Methods

This preliminary cross sectional and non-interventional study was conducted during year 2012-2013.Blood was collected from patients who attended the Out-Patients Department (OPD) of Diabetic clinic of R. G. Kar Medical College & Hospital, Kolkata, West Bengal. 92 patients whose FPG >126 mg/dl (12)were considered as cases. Patients were included provided they had no known hemolytic disorder or any condition which may lead to hyperglycemia other than Type 2DM, had attained the age of 18 years and could give informed consent after understanding the objectives of the study. Written consents were obtained from patient. None of the patients were suffering from any genetic disorder other than type 2 DM, somatic illness, neoplasm, metabolic or endocrine disorder or neurological diseases. None of the patient use of alcohol or nicotine in any form, psychoactive substance abuse or dependence disorder. None of them were suffering from renal failure-excluded by measuring serum urea, creatinine(13).Those subjects was not fulfilled our including criteria were excluded from this study.A group of 107 age and sex matched healthy individuals with no history of diabetes mellitus served as controls.As participants attended the hospital OPD from a large rural base, they had approximately similar ethnicity, socioeconomic status and dietary habits.Above mentioned diseases were excluded from both case and control groups.

Fasting blood was collected by venipuncture for the determination of different biochemical parameters. The blood samples were subjected to centrifugation at 3,000 rpm for 10 min for separation of plasma. The plasma thus obtained was analysed for biochemical parameters such as plasma glucose and glycatedhemoglobin. These were measured with the kits provided by Coral biosystems.Fasting plasma glucose was determined by glucose oxidase –peroxidase method(14) using kits provided by Crest Biosystems, Goa, India. Within assay precision of Glucose (GOD/POD) method were determined by analysis control materials (BIORAD Lot No.14190 coefficient of variance-4.78% in level 1 control).

Glycatedhemoglobin was determined by ion exchange resin method using kits provided by Crest Biosystems, Goa, India.GlycatedHemoglobin has been defined as fast fraction hemoglobins HbA1 (HbA1a, HbA1b, HbA1c) which elute first during column chromatography. The non – glycatedHb, which consists of the bulk of Hb , has been designated as HbA0(15).A hemolysed preparation of whole blood (collected in EDTA vial) is mixed continuously for 5 minutes with a weakly binding cation exchange resin. The labile fraction is eliminated during the hemolysate preparation and during the binding. During the mixing, HbA0 binds to the ion exchange resin leaving GHb free in the supernatant. After the mixing period, a filter separator is used to remove the resin from the supernatant. The percentglycatedHb is determined by measuring absorbance (405nm) of the glycatedHb (GHb) fraction &The total Hb fraction. The ratio of the absorbance of the glycatedHb (GHb) fraction & total Hb fraction of the control and the test is used to calculate the percentglycatedHb.

Data entry was done right after collection of relevant data for a given patient was completed. Data analysis was done after completion of data collection for all patients. SPSS software was used for data analysis.

III. Results

In this study we included 199 subjects (N=199), male: 109 and female: 90. This difference is not statistically significant (chi-square = 3.258, df = 1, p = 0.0711). Mean age of this study population is 38.21yrs. After applying the existing criteria for the diagnosis of diabetes mellitus we found that 107 subjects are non diabetic and the rest 92 subjects are diabetic patient. Descriptive with Students t test statistics of these two groups are as follows. In our study FPG (mg/dl) were significantly increased in cases (185.70 ±56.58) in comparison to controls (96.37±21.37) (t=15.12; p = <0.001) (table no 1).FPG values were >126mg/dl in all subjects in diabetic group.HbA1C(%) were significantly increased in cases (8.14±1.81) in comparison to controls (5.05 ±0.82) (t=15.85; p = <0.001) (table no 1)ROC (receiver operating characteristics) curve was performed between existing criteria for the diagnosis of diabetes mellitus (fasting plasma glucose > 126 mg/dl) and level of GHbA1$_C$ to find out that >6.5% of GHbA1$_C$ is diagnostic of diabetis mellitus or not.

From this we can say that if >6.5% of GHbA1$_C$ is used for the diagnosis the sensitivity and specificity would be about 92% and 91% respectively. To establish an equation for determining estimated average glucose (eAG) concentration we assume HbA1$_C$ as predictors/constant and plasma glucose as dependant variable. After doing the regression analysis we found the following:

Hence from this regression study we can establish the equation as follows:

Average glucose (eAG) concentration (mg/dl) = 28.184*HbA1$_C$ – 45.055

IV. Discussion

The revised criteria for the diagnosis of DM emphasize the FPG as a reliable and convenient test for diagnosing DM in asymptomatic individuals. A random plasma glucose concentration >11.1 mmol/L (200 mg/dL) accompanied by classic symptoms of DM (polyuria, polydipsia, weight loss) is sufficient for the diagnosis of DM. Oral glucose tolerance testing, although still a valid mechanism for diagnosing DM, is not recommended as part of routine care. There is a need to simplify screening tests for type 2 diabetes mellitus (Type 2DM) so patients can be identified earlier and more efficiently. Glycated haemoglobin (HbA1c) is to use in the diagnosis of diabetes, based on its lower day-to-day variability and considered the 'gold standard' for monitoring metabolic control in diabetes. An International Expert Committee recently recommended HbA1c as a better method than measurement of glucose due to ease of use: not necessarily in the fasting state. The HbA1c result reflects longer term glycaemia and is less affected by recent physical/emotional stress. The term GLYCATION is suggested & currently accepted by the IFCC / IUPAC for all such nonenzymatic reactions that link a sugar to a protein or peptide. In a normal person glucose circulates in the blood. The erythrocytes are freely permeable to glucose. There is proportionate elevation of glucose level in erythrocytes when the same increases in the plasma. When glucose is in its free aldehyde form, it can react with the hemoglobin molecule present in the erythrocytes. The free aldehyde group of the glucose attaches to one or both of the N-terminal valine of the β chain or the free amino group of lysine of the hemoglobin molecule to form a Schiff's base or an aldimine. This process is relatively fast and reversible. It depends on the glucose concentration in the erythrocytes. As the glucose molecule has a hydroxyl group at carbon-2, it can undergo a shift in the double bond to carbon-2 via what is known as an Amadori rearrangement to form a keto –amine. This is a slow process and once formed is relatively irreversible. The stable ketoamine form, with the glucose molecule attached to the hemoglobin remains for the life span of the erythrocyte. This process is called Glycation. In this study we included 199 subjects (N=199), male: 109 and female: 90. This difference is not statistically significant (chi-square – 3.258, DF – 1, p - 0.0711). Mean age of this study population is 38.21yrs, among them mean value of HbA1C was 6.59% and plasma glucose was 141.04mg/dl.After applying the existing criteria for the diagnosis of diabetes mellitus we found that 107 subjects are non diabetic and the rest 92 subjects are diabetic. Estimated average plasma glucose was 185.70mg/dl in diabetic individual and 96.37mg/dl in nondiabetic individual. It was significantly increased (t value=15.127, p value=<0.001) in diabetic individual in comparison to nondiabeticindividual.We also found mean of HbA1C was 8.15% in diabetic individual and 5.05% in nondiabetic individual. It was significantly increased (t value=15.854, p value = <0.001) in diabetic individual in comparison to nondiabeticindividual.From ROC curve we found that if we take glycated cut off as 6.5%, for diagnosis of DM then sensitivity 92%, specificity 91%.

From regression analysis we may predict average estimated plasma glucose from HbA1C level

$$\text{Average glucose (eAG) concentration (mg/dl)} = 28.184 * HbA1_C - 45.055$$

So, we can conclude that HbA1c >/=6.5% is corroborative with the existing diagnostic criteria (FPG>126mg/dl) of diabetes mellitus. We can estimate average blood glucose from HbA1c. It can be useful as simple screening tests for type 2 diabetes mellitus (Type 2DM) so patients can be identified earlier and more efficiently.

Limitations of the Study:
➢ Due to time restraint this study could not be extended to a larger population.
➢ Food habits, life style, body mass index (BMI) and lipid profile parameters were not taken into consideration.
➢ A more detailed follow up after diagnosis and initiation of therapy was beyond the scope of the present study.

A more planned prospective study with greater control over confounding variables is needed for generating stronger evidences.

Bibliography

[1]. Powers AC. Diabetes Mellitus. In: Fauci AS, Kasper DL, Longo DL, Braunwald E, Hauser SL, Jameson JL, et al., editors. Harrison's Principles of Internal Medicine 17th ed: The McGraw-Hill Companies; 2008. p. 2275-304.
[2]. Buse JB, Polonsky KS, Burant CF. type 2 diabetes mellitus. In: Kronenberg HM, Melmed S, Polonsky KS, Larsen PR, editors. Williams Textbook of ENDOCRINOLOGY11th ed: SAUNDERS ELSEVIER; 2008. p. 1329-58.
[3]. Anjana R, Ali M, Pradeepa R, Deepa M, Datta M, Unnikrishnan R. The need for obtaining accurate nationwide estimates of diabetes prevalence in India - rationale for a national study on diabetes. The Indian journal of medical research. 2011 Apr;133:369
[4]. IDF Diabetes Atlas Update2012.
[5]. Kaveeshwar SA, Cornwall J. The current state of diabetes mellitus in India. Australas Med J. 2014 Jan 31;7(1): 45-8.
[6]. GUPTA R, MISRA A. Type 2 diabetes in India: regional disparities. Br J Diabetes Vasc Dis. 2007;7:12-6.
[7]. Abstracts of the 64th Scientific Sessions of the American Diabetes Association. 2004 June 4-8.
[8]. SA M, MJ D, BT S, ME C, D W, K K. Should glycated haemoglobin (HbA1c) be used to detect people with type 2 diabetes mellitus and impaired glucose regulation? Postgrad Med J. 2010;86(1021):656-62.

[9]. A L, A M, D F. The general use of glycated haemoglobin for the diagnosis of diabetes and other categories of glucose intolerance: still a long way to go. Nutr Metab Cardiovasc Dis. 2011 21(7):467-75.
[10]. Borg R, Nerup J, Nathan D, Kuenen J, Zheng H, Schoenfeld D. Glycated haemoglobin may in future be reported as estimated mean blood glucose concentration--secondary publication. Ugeskr Laeger. 2009;171(45):3262-5.
[11]. RAMACHANDRAN A, SNEHALATHA C. Current scenario of diabetes in India. Journal of Diabetes 1. 2009:18-28.
[12]. American Diabetes A. Standards of medical care in diabetes--2013. Diabetes Care. 2013 Jan;36 Suppl 1:S11-66.
[13]. Mostafa S, Davies M, Srinivasan B, Carey M, Webb D, Khunti K. Should glycated haemoglobin (HbA1c) be used to detect people with type 2 diabetes mellitus and impaired glucose regulation? Postgrad Med J. 2010;86(1021):656-62.
[14]. Trinder P. Determination of blood glucose using 4-amino phenazone as oxygen acceptor. J Clin Pathol. 1969 Mar;22(2):246.
[15]. Trivelli LA, Ranney HM, Lai HT. Hemoglobin components in patients with diabetes mellitus. N Engl J Med. 1971 Feb 18;284(7):353-7.

Table No1: Comparison of mean value of different parameters between cases and controls

Group		N	Mean	Std. Deviation	't' value	Significance
Age	Non diabetic	107	37.12yrs	11.549		
	Diabetic	92	39.30yrs	11.153	1.351	0.178
HbA1$_c$	Non diabetic	107	5.0521%	0.82237		
	Diabetic	92	8.1476%	1.81525	15.854	<0.001
Plasma Glucose	Non diabetic	107	96.37mg/dl	21.376		
	Diabetic	92	185.70mg/dl	56.583	15.127	<0.001

Table No1 showed plasma glucose and HbA1C significantly elevated among diabetic group in comparison to non diabetic. There is no significant difference in age among two group.

Figure No: 1 ROC curve between diabetes mellitus and level of HbA1$_C$
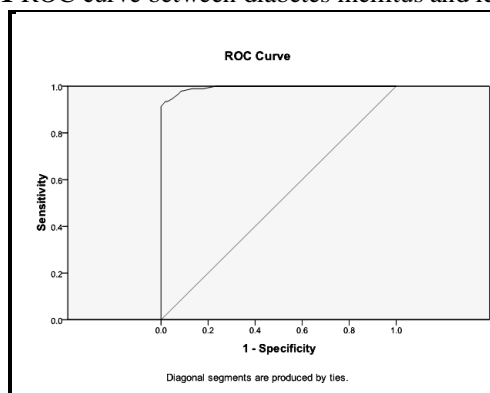
Coordinates Of RocGhba1$_c$Sensityvity 1-Specificity

6.1000	.978	.084
6.2250	.967	.075
6.3500	.946	.047
6.3950	.935	.028
6.4350	.935	.019
6.5550	.913	.000
6.6800	.870	.000
6.7250	.859	.000
6.7700	.804	.000
6.8500	.772	.000
6.8950	.739	.000
6.9400	.728	.000
7.0100	.717	.000

From figure no 1:ROC curve showed that glycated cut off may be taken as 6.5%, for diagnosis of DM then sensitivity 92%, specificity 91%.

Table 2: regression analysis

Coefficients[a]						
Model		Unstandardized Coefficients		Standardized Coefficients	't'	Sig.
		B	Std. Error	Beta		
	(Constant)	-45.055	4.176		-10.788	<0.001
	HbA1$_c$	28.184	.614	.956	45.910	<0.001
a.	Dependent Variable: Plasma glucose.					

From regression analysis average estimated plasma glucose may be predicted from HbA1C level

Average glucose (eAG) concentration (mg/dl) = 28.184*HbA1$_C$ – 45.055