

RELIABLE MARKER OF ATHEROGENICITY IN TYPE 2 DM – A COMPARATIVE STUDY ON GLYCOSYLATION GAP AND HAEMOGLOBIN GLYCATION INDEX.

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Abstract

Background: To prevent acute and chronic complications, monitoring & controlling the level of diabetic indicators become an integral part of diabetic care. Factors that increase or decrease the intracellular glucose level relative to external plasma glucose levels causes the alteration in glycation, which leads to discordance between HbA1C and fructosamine. Aim of the study was to compare the Cardiovascular disease(CVD) risk prediction property of GG(Glycosylation gap) & HGI (Haemoglobin glycation index), 2 statistical measures to assess the disparity between actual HbA1C and the predicted value of HbA1C in Diabetes Mellitus(DM),by correlating them with predictor of atherogenicity and CVD– Atherogenic index of plasma(AIP).

Materials & Method: This Cross sectional study conducted on 142 type 2 DM patients .Blood samples collected to estimate FBS, HbA1c, Fructosamine , Triglyceride & HDL. GG & HGI calculated from predicted HbA1C from fructosamine and Predicted HbA1C from mean blood glucose respectively.

Results: AIP&HGI showed significant correlation compared to GG and AIP. Study concludes the usefulness of HGI in type 2 DM as a regular monitor for CVD risk prediction for improved healthcare.

Keywords: Haemoglobin glycation index ; Glycosylation gap ;Cardiovascular disease.

Introduction

Among all Non communicable diseases Diabetes, “the honey urine disease” causes 5% of all death¹. Uncontrolled sugar level increases long term vascular complications of diabetes such as coronary artery disease, stroke, heart attack, kidney failure, blindness, heart failure and neuropathy². Among that 50% of people with Type 2 Diabetes Mellitus (DM) die of cardiovascular diseases¹.In order to prevent acute and chronic complications, monitoring & controlling the level of diabetic indicators become an integral part of diabetic care. Measurement of Advanced Glycation End products (AGE) is an indirect indicator of risk for complications in DM.

Maillard reaction is the process by which carbonyl group of glucose react nonenzymatically with amino group in proteins ,lipids & nucleic acid to form aldimines or early glycation products (Schiff's base).These early glycation products consequently undergo the amadori rearrangement & form intermediate glycation products like HbA1C & fructosamine^{3,4}

HbA1C assay become the benchmark in assessing chronic glycemia in research & management. Maintaining it within normal limits has been shown to reduce long term complications⁵. Fructosamine, first described in 1982⁶ also correlate well with mean blood glucose value⁷.Several

studies revealed that, these markers plays an important role in predicting the complications of DM.

Mean blood glucose and fructosamine would reflect the extracellular glucose environment. Factors that increase or decrease the intracellular glucose level relative to external plasma glucose levels causes the alteration in glycation, which leads to discordance between HbA1C and fructosamine^{8,9}. To address this discrepancy between mean blood glucose and HbA1C, the measurement of glycation gap has been proposed (GG)^{8,10,11}. Another statistical measure to assess the disparity between actual HbA1C and the predicted value of HbA1C based on plasma glucose levels has been developed and termed Haemoglobin glycation index (HGI)¹²⁻¹⁵. Both GG and HGI can predict the risk of development of diabetic complications like CVD, retinopathy, nephropathy etc.^{14,16,17,18}.

A comparative study on GG & HGI in type 1 DM patients showed that they are highly correlated in both direction and magnitude¹⁹.There are several studies which explains the relationship between GG,HGI,& diabetic complications. But there is paucity of information regarding the comparison of GG& HGI in predicting CVD risk, which is the cause for highest death rate in Type 2 DM patients.

The atherogenic index of plasma (AIP) value, which is derived by the logarithmic transformation of the number

found by dividing plasma TG value to HDL value, can be a good marker for the risk of atherosclerosis and cardiovascular disease²⁰⁻²⁵

The aim of this study is to find out the reliable and better predictor of CVD in Type 2 DM among GG & HGI by correlating AIP with GG & HGI, which will help in early diagnosis and management of cardiovascular risk in subjects with type II DM.

Aims

Aim of this study was to compare the Cardiovascular disease(CVD) risk prediction property of GG(Glycosylation gap) & HGI(Haemoglobin glycation index), 2 statistical measures to assess the disparity between actual HbA1C and the predicted value of HbA1C in Diabetes Mellitus(DM), by correlating them with predictor of atherogenicity and CVD—Atherogenic index of plasma(AIP).

Materials and methods

Study subjects:

Type 2 diabetes mellitus patients of age >40 years attending the general medicine OPD of the institution who fulfils the inclusion criteria during the study period. Institutional ethical committee clearance obtained to carry out the study.

Inclusion criteria:

Type 2 diabetes mellitus patients of minimum 1 year duration of age >40yrs years attending the general medicine OPD of the institution

The Type 2 DM Subjects were included for the study as per the guidelines of WHO 2006 (FBS >126 mg/dl) and/or ADA 2010(HbA1c >6.5%)

Exclusion criteria: subjects having the following conditions were excluded from study

1. Undergoing treatment for thyroid disorders
2. Taking lipid lowering drugs
3. Treatment for cancer
4. known case of ischemic heart disease
5. known case of chronic liver disease
6. On diuretics
7. On Oral contraceptive pills
8. History of hypoglycemic episode in the past three months
9. With severe hyperglycemia (FBS >300MG%)

This cross sectional study was conducted for a period of 18 months .Sample size was calculated to be 142 by convenience sampling technique .Informed written consent taken from all subjects. Subject's clinical history and details taken according to standard proforma. Subjects examined for general physical health.5ml of fasting blood sample collected under universal precautions. Whole

blood for HbA1C by turbidimetric inhibition assay. Serum separated after centrifugation and used for analysis of following parameters.

Fasting blood glucose by enzymatic hexokinase method

Fructosamine estimation by Nitro blue tetrazolium method in fully automated analyser

Serum triglycerides by enzymatic GPO-PAP method.

Serum HDL cholesterol by Direct enzymatic method

Glycosylation gap calculated by equation

GG = HbA1c – predicted HbA1C from fructosamine²⁶

Haemoglobin glycation index calculated by equation

HGI = HbA1C – Predicted HbA1C from mean blood glucose²⁶

Statistical analysis done using SPSS software, multiple tables, scatter plot & graphs were used to represent the result.

Results

Table I. Baseline characteristics of study subjects

	Mean ±SD
AGE	55.88 ±10.018
DURATION OF DIABETES (yrs)	7.51 ±5.993
FBS (mg/dl)	188.297 ±56.407
HbA1C (%)	8.96 ±1.748
FRUCTOSEAMINE(μmol/L)	387.125 ±91.184
TG(mg/dl)	229.494 ±146.78
HDL(mg/dl)	41.411 ±13.707
Log TG/HDL (AIP)	.700 ±.271
GG	.005 ±1.204
HGI	.123 ±1.601

Table I shows the baseline characteristics of study subjects. Mean age of the study population was 55.88 ±10.018. Mean duration in diabetes was 7.51 ±5.993. Mean FBS, HbA1C and fructosamine were 188.297 ±56.407, 8.96 ±1.748, 387.125 ±91.184 respectively. All the glycemic control parameters were in the higher level as the study population is Type 2 DM patients of minimum 1 year duration.

Mean atherogenic index of plasma was .700 ±.271, which is considered high risk^{26,28}. The mean GG and HGI calculated from predicted HbA1C by using equation from linear regression analysis was (figure I & II) .005 ±1.204 & .123 ±1.601 respectively. Equation predicted HbA1C for calculation of GG & HGI derived from this are HbA1C = .014 x Fructosamine + 3.54 & HbA1C = 0.020 x FBS + 5.151 respectively.

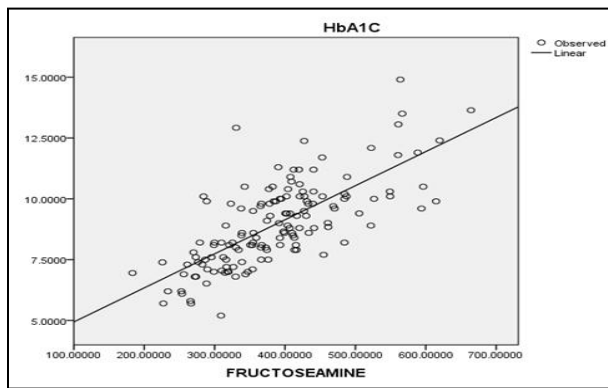


Figure I: linear regression analysis of Fructosamine and HbA1C

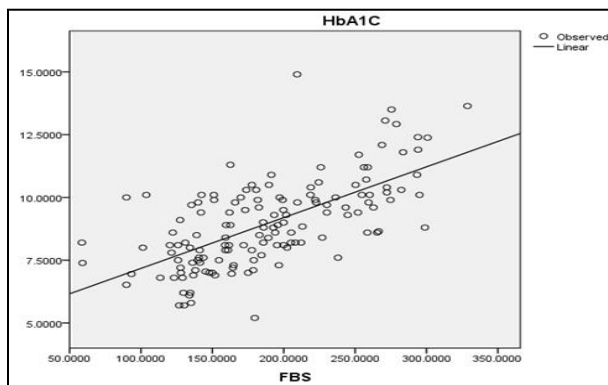


Figure II: linear regression analysis of FBS and HbA1C

Table II: Correlation analysis between atherogenic index of plasma (AIP) with GG & HGI

		HGI	GG
logTG/HDL (AIP)	Pearson Correlation	.181*	.097
	Sig. (2-tailed)	.031	.252
	N	142	142

*. Correlation is significant at the 0.05 level

Table II shows correlation analysis between atherogenic index of plasma with GG and HGI .It shows HGI is correlating significantly (Pearson correlation .181 & 2 tailed significance .031)with AIP.AIP and glycosylation gap showed a Pearson correlation .097 with 2 tailed significance of .252 ,which is less significant when compared with HGI.

Discussion

Patients with diabetes mellitus are at risk for developing severe debilitating complications, including cardiovascular diseases,retinopathy, nephropathy, neuropathy and stroke²⁷. Randomized trials have documented that, lowering haemoglobin A1c (Hb A1c)concentrations significantly reduces the onset and rate of progression of microvascular complications²⁶.

The discordance in the measurement of HbA1C which is measured intracellularly and plasma proteins (Fructosamine) measured extracellularly is limiting the use of them to predict the complications in diabetes mellitus. Exposure of Haemoglobin to glucose cause difference in the haemoglobin glycation relative to blood glucose might be due to differences in the permeability of RBCs to glucose⁹.

The statistical measures like Glycosylation gap and Haemoglobin glycation index will have a better role in predicting macro and microvascular complications in diabetes mellitus than glycaemic control markers as they are derived from HbA1C value predicted from blood glucose and fructosamine.

In our study Mean age of the study population was 55.88 ±10.018 .Mean duration of diabetes for the population was 7.51 ±5.993(Table I).In a study by Van steen et al²⁷ Patients in the high HGI subgroup were significantly younger, had a longer duration of diabetes(7.8 years in the high versus 6.7 years in the intermediate and 5.4 years in the low subgroup).Duration of diabetes is important as it is an important factor in predicting the complications in diabetes.

Glycaemic control markers ,HbA1C & fructosamine was 8.96 ±1.748 & 387.125 ±91.184 respectively. Linear regression analysis showed a positive correlation of FBS and Fructosamine with HbA1c(figure I &II $R^2 = .426,534$ respectively). Study by Mee Kyoung et al used Glycated albumin (GA) instead of fructosamine for glycosylation gap calculation (predicted HbA1C = .146 x GA + 4.772)²⁸.

Atherogenic index of plasma calculated as log TG/HDL was .700 ±.271.It is in the high risk category for cardiovascular disease²⁹.Pearson correlation of AIP with Glycosylation gap and HGI showed that there is correlation in both the parameters but a significant correlation was shown by HGI when compared to GG(Table II) . This agree with the study by Chang Ho Ahn et al¹⁸which showed a higher HGI was significantly associated with macrovascular complications even after adjusting for traditional CVD risk factors and HbA1c levels. This suggests that the HGI might have an additional impact on macrovascular complications beyond the HbA1c level. Several mechanisms might mediate this association between high HGI and macrovascular complications.

It is also supported by a study by Cohen et al showed that glycosylation gap could explain the excess interindividual variation in HbA1C also it can predict the progression of nephropathy⁸.Also in the action to control cardiovascular risk in diabetes trial ,subjects with high HGI had higher incidence of CVD¹⁶.

Study by Maria et al linear regression analysis model including several atherosclerotic risk factors also showed

HGI was the major predictor of IMT(intima medial thickness) & logistic regression analysis adjusted for confounders, individuals with high HGI showed a 2.7-fold increased risk of vascular atherosclerosis as compared with subjects with low HGI³⁰.

So haemoglobin glycation index, simple statistical measure calculated from HbA1C and mean blood glucose level, can be used to predict the CVD risk in type 2 Diabetes mellitus patients as a regular monitor during routine check up & help the clinician in both monitoring and management of the disease.

As a continuation of this study we will be conducting further studies to know the better predictor among GG& HGI in various other complications of Diabetes Mellitus and their association with severity of complications.

Conclusion

Statistical measures like GG and HGI has a better role in predicting the most important complication of diabetes like CVD, as there is a discordance in extracellular and intracellular glucose value measured by Fructosamine and HbA1c. This study concluded that there is a significant correlation between HGI and atherogenicity predictor index AIP in type 2 Diabetes mellitus patients when compared to GG projecting its usefulness as CVD risk predictor in Type 2 DM.

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