Original Article

Glycosylation Gap in Patients with Diabetes with Chronic Kidney Disease and Healthy Participants: A Comparative Study

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Abstract

Aim: The aim of this study it to determine the level of glycosylation gap in patients with type 2 diabetes and its relation with kidney dysfunction. **Materials and** Methods: In this study, 150 individuals were enrolled (aged 20–75 year) and divided into three groups. Group 1 included 50 nondiabetic individuals who served as control. Group 2 included 50 patients with type 2 diabetes without chronic kidney disease (CKD), and in Group 3, there were 50 patients with type 2 diabetes with CKD. Glycated hemoglobin (HbA1c) and fructosamine (FA) were measured in all groups to determine the glycosylation gap (GG), predicted HbA1c, and mean blood glucose (MBG). GG is defined as the difference between measured HbA1c and HbA1c predicted from FA based on the population regression of HbA1c on FA. The variables were compared by correlation analysis. **Results:** Serum creatinine level was significantly high in patients with CKD (1.93 ± 0.99) as compared to patients with diabetes and control (0.891 ± 0.16; 0.912 ± 0.1), respectively. The study demonstrated a significant elevation in serum FA, measured HbA1c and predicted HbA1c, MBG in patients with diabetes with CKD as compared with those of without CKD, and controls. GG was found in healthy control (0.51 ± 0.78), patients with type 2 diabetes without CKD (0.62 ± 0.45), and patients with diabetes with CKD (1.0 ± 0.91), respectively. **Conclusion:** It is concluded that GG may be a useful clinical research tool for evaluating pathological source of variation in diabetes complications such as kidney disease.

Keywords: Chronic kidney disease, diabetes mellitus, fructosamine, glycated hemoglobin, glycosylation gap

Introduction

Diabetic nephropathy progressively affecting up to a third of patients with diabetes mellitus, a resource-consuming complication of diabetes and is the most common cause of renal failure. Although we have better knowledge related to this complication, the intimate mechanisms leading to the development and progression of renal injury are not exactly clear.[1] Hyperglycemia is responsible for the succession of diabetic nephropathy through oxidative stress, renal polyol formation, mitogen-activated protein kinases, protein kinase C, and accumulation of early and advanced glycosylation end products (AGEs).[2] Glycation is continuously occurring process in diabetes mellitus targeting hemoglobin and serum proteins. It is a nonenzymatic process in which free aldehyde group of glucose and amino groups of proteins allocated and formed Schiff base then reorganized in a stable ketoamine, Amadori adduct. After this, ketoamine adduct converts into AGEs.[3] In fact, nonenzymatic glycation has been strongly related to hyperglycemic conditions and therefore, to chronic complications associated with diabetes mellitus and renal failure, as well as crumble changes occurring in the course of aging.[4] Glycated hemoglobin (HbA1c), an Amadori product of nonenzymatic glycation, is regarded as the gold standard for the measurement of glycemic control and very useful in the management of diabetic complications.[5,6] Despite the utility and invaluability of HbA1c, clinicians still encounter discordance between HbA1c and other measures of glycemic control in their patients.[7,8] On the other hand, HbA1c is also not considered a reliable marker due to longer life span of erythrocytes and in those who have hemoglobin metabolism-related diseases. Fructosamine (FA), an Amadori adduct, refers to all glycated serum proteins included glycated

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albumin (GA); it is a measure of average glycemic control over a shorter period (2–3 weeks).[3] Discrepancies between HbA1c and FA measure of glycemic controls are commonly encountered. A number of mechanisms might explain the discordance between HbA1c and FA, but whether or not they are consistent is uncertain. However, the discrepancy between HbA1c and FA cannot be ignored in monitoring of glycemic control. To address this discrepancy, glycosylation gap (GG) may be a useful clinical research tool. GG has been defined as the difference between measured HbA1c and HbA1c predicted from another measure of glycemic control like FA, based on the population regression of HbA1c on FA.[9] In a more recent clinical research study, GG was found to be a better indicator than HbA1c for assessing the risk of death and hospitalization in diabetic dialysis patients.[10]

The aims of this statistical analyses study were to estimate GG values in healthy participants, diabetes without chronic kidney disease (CKD), and diabetes with CKD patients. Further, it was extended to establish the correlation between HbA1c and FA levels in patients with diabetes with CKD.

Materials and Methods

HbA1c kit was purchased from BioRad, USA. Creatinine kit was purchased from Avantor Performance Materials India Limited, Dehradun. Cholesterol reagent was procured from Pointe Scientific Inc., MI, USA. Vacutainers were obtained from BD Bioscience. All other reagents and chemicals used were of the highest analytical grade available.

Study design

One hundred and fifty patients with type 2 diabetes with or without CKD aged 20–75 years who attended outpatients' clinic and indoor in Rajiv Gandhi Centre for Diabetes and Endocrinology, J.N. Medical College Hospital, Aligarh, India, were included in this study. All participants are divided into three groups, each of 50 participants with sex-matched as follows.

Group 1 – 50 nondiabetic participants as control
Group 2 – 50 patients with diabetes without CKD
Group 3 – 50 patients with diabetes with CKD.

The diagnosis of diabetes was made on the basis of the American Diabetes Association (ADA), i.e., fasting plasma glucose ≥126 mg/dl and 2 h postprandial plasma glucose ≥200 mg/dl. Healthy participant without diabetes serves as control. A detailed history and physical examination was done in all the participants enrolled. All the participants were evaluated for age, duration of diabetes, blood glucose, HbA1c, and blood pressure.

Inclusion criteria

- Patients with type 2 diabetes mellitus (T2DM) aged 25–75 years
- The diagnosis of diabetes was made on the basis of the guideline laid down by the ADA criteria (2010)
- Hypertensive
- Possible CKD (any stage) diagnosed in patients with T2DM.

Exclusion criteria

- Type 1 diabetes mellitus and gestational diabetes
- Mental illness
- Pregnant and lactating females
- Inflammatory or infectious diseases
- Rheumatic, cancer, hematological diseases.

Ethical approved

The present study was carried out in Rajiv Gandhi Centre for Diabetes and Endocrinology, Aligarh Muslim University, Aligarh, India, and approved by the ethical committee of J.N. Medical College, Aligarh. Before enrolment in the study, informed consent of the patients to use their clinical data for research was obtained.

Clinical analysis

Blood glucose (fasting and postprandial) was measured by glucose oxidase-peroxidase enzymatic method. HbA1c estimation was done by high-performance liquid chromatography method. Serum creatinine was calculated by Jaffe/Kinetic method. FA level was measured according to the method of Johnson *et al*., 1982. Briefly, sample (200 µl) was added to 96 well microtiter plates in duplicate followed by 100 µl of nitroblue tetrazolium reagent (250 µmol/L in 0.1 mol/L carbonate buffer, pH 10.35) was added to each well and incubated at 37°C for 2 hrs. The color was read at 525 nm on a microplate reader. The purple color monoformazan was quantitated using an extinction coefficient of 12,640 cm^{-1}/mol.

Mathematical analysis

Mean blood glucose (MBG), predicted HbA1c, and GG were calculated using equations. MBG was calculated using the equation:[11]

$$MBG=1.76\times(HbA1c)-3.67 \text{ mmol/L} \quad (1)$$

HbA1c predicted was computed from the regression equation.

$$pHbA1c = 0.017\times FA+1.61 \quad (2)$$

GG from each individual was calculated by the method of Cohen *et al.*

$$GG=\text{Measured HbA1c-Predicted HbA1c} \quad (3)$$

According to this formula, GG is positive if the measured HbA1c is greater than the predicted from FA values, and if GG is negative than measured, HbA1c is less than HbA1c predicted. GG is 0 when HbA1c and FA are concordant.[9]

Statistical analysis

All data are expressed in terms of mean ± standard deviations. The baseline parameters were compared in all three groups by one-way ANOVA analysis. *t*-test was applied to compare measured HbA1c and FA in each test group and control group. In all tests, $P < 0.001$ was considered statistically significant. All statistical analyses were conducted using the software SigmaPlot 13.0 version (Copyright Systat Software, Inc).

Table 1: Baseline and biochemical variables in patients with diabetes without and with chronic kidney disease and controls

Parameter	Healthy control	T2DM	T2DM + CKD	*P* (one-way ANOVA)
Age (year)	50.06±4.7	52.84±8.2	51.28±9.4	NS
Duration of diabetes	-	3.25±2.9	8.44±6.1	<0.001
Weight (kg)	60.32±8	63.28±9.2	60.03±12	NS
Height (cm)	161.84±9.8	166±9	161.7±8.6	0.135
BMI (kg/m^2)	23.16±2.2	25.88±4.1	24.3±3.8	NS
Systolic BP (mmHg)	130±15.2	135.92±18.2	148.34±20.2	<0.001
Diastolic BP (mmHg)	82.23±6.7	85.42±9.6	87.73±9.7	<0.001
Fasting plasma glucose (mg/dl)	86.98±11.3	128.52±44.0	131.88±44.5	<0.001
Postprandial glucose (mg/dl)	122.94±11.4	183.64±52.5	212.7±73.6	<0.001
HbA1c (%)	4.89±0.66	7.04±0.92	9.8±2.5	<0.001
Serum creatinine (mg%)	0.891±0.16	0.912±0.1	1.93±0.99	<0.001

Data are mean±SD. BMI: Body mass index, BP: Blood pressure, NS: Not significant, SD: Standard deviation, HbA1c: Glycated hemoglobin, T2DM: Type 1 diabetes mellitus, CKD: Chronic kidney disease

Results

The baseline characteristics and analytical parameters of the all three groups are summarized in Table 1. Serum creatinine level in patients with CKD was found significantly high in comparison to patients with diabetes without complications and healthy participants. The results of this study demonstrated a significant elevation in GG, MBG, serum FA, and measured and predicted HbA1c in patients with type 2 diabetes with CKD as compared to control individuals. GG values were found in control, patients with type 2 diabetes without CKD, and patients with type 2 diabetes with CKD (0.51 ± 0.7, 0.62 ± 0.4, 1.0 ± 0.9, $P \leq 0.001$), respectively. FA concentration was found high in patients with diabetes without and with CKD (315 ± 35.3; 382.4 ± 60.3) as compared to healthy controls (248.68 ± 24.54). All values are shown in Table 2. Furthermore, a correlation was evaluated between HbA1c and FA in all three groups. As shown in Figure 1, control group showed no relation between HbA1c and FA ($r^2 = 0.04$), but in Figures 2 and 3, T2DM without CKD and T2DM with CKD showed positive correlation of HbA1c with FA ($r^2 = 0.45$, 0.76, respectively). Table 3 showing the data of correlation analysis between different variables and glycosylation gap in patients with CKD. The degree of correlation is very similar to previous reports.[12-15]

Table 2: Mathematical measurements in control and patients with diabetes without and with chronic kidney disease

Parameters	Healthy control ($n=50$)	T2DM ($n=50$)	T2DM + CKD ($n=50$)
Measured HbA1c	5.18±0.57	7.04±0.9	10±2.4
Predicted HbA1c	5.83±0.41	6.7±0.63	8.1±1.02
Fructosamine (μmol/L)	248.68±24.54	315±35.3	382.4±60.3
Mean blood glucose	5.45±1.01	9.0±1.5	12.4±2.9
Glycosylation gap	−0.51±0.7	0.622±0.45	1.0±0.92

HbA1c: Glycated hemoglobin, T2DM: Type 1 diabetes mellitus, CKD: Chronic kidney disease

Discussion

Elevation of HbA1c, FA, and GG was observed in patients with type 2 diabetes with CKD compared with control group. The risk of secondary complications in patients with type 2 diabetes is highly associated to the chronic level of blood glucose. Nonenzymatic glycosylation is accelerated in hyperglycemic conditions and chronic complications related to diabetes.[4] Diabetes Control and Complications Trial (DCCT) reported that to prevent the progression of diabetic complications, it is very necessary to reduce and maintain blood glucose levels under the physiological range. Hence, it is important to monitor the blood glucose level time to time and to prevent the further processing of diabetes complications, has led to the widespread use of a marker which can give the short time glycemic status of patients such as FA and GA rather than HbA1c. FA level is an alternative test to screen the patients with diabetes, but the data are limited.[16] The problem is with the large divergences between HbA1c and FA estimations in the assessment of glycemia. Cohen *et al*. proposed that the measurement of GG can be a useful clinical tool for evaluating physiologic sources of variation in the diabetic complications beyond glycemic control. GG improves the quality of the monitoring of glycemic control, especially for those patients whose HbA1c levels do not truly reflect the mean glucose level.[17]

In the present study, the glycemic status of patients with T2DM with CKD is compared with T2DM without any complication and healthy

control. The mean fasting blood glucose and postprandial glucose were increased in T2DM and T2DM with CKD in respect to control group. On the other hand, duration of diabetes, serum creatinine, blood pressure, and GG were also significantly increased in diabetic CKD group. Serum FA level is highly correlated to the level of HbA1c. It gives information of glycemic control that lasts 2–3 weeks and HbA1c reflect glycemic control lasting 4–6 weeks. Reduction in HbA1c and FA to the normal range has the lowest risk for the secondary complications such as nephropathy.[18] Cohen *et al*. have reported that HbA1c and FA are highly correlated in the

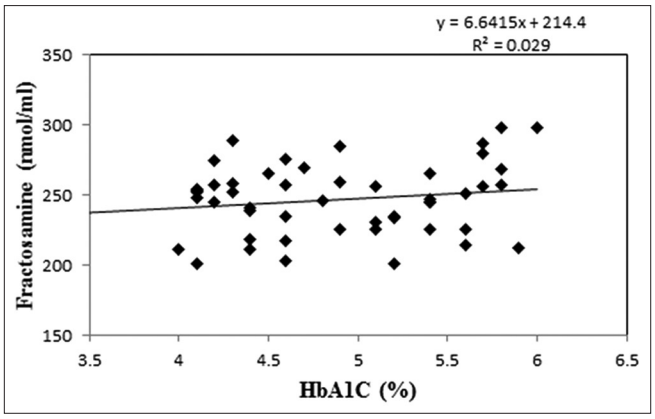

Figure 1: Relationship between percent glycosylation of hemoglobin with fructosamine in control group

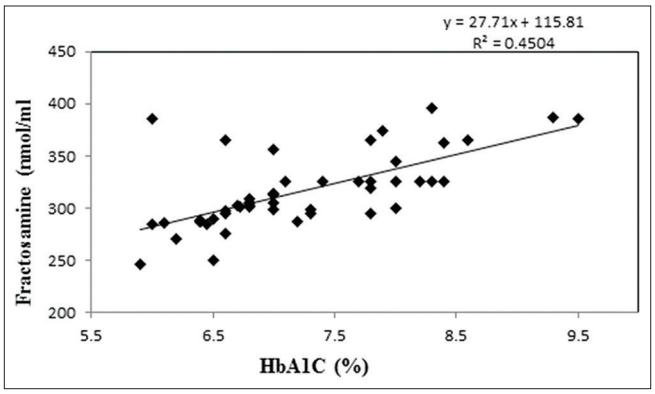

Figure 2: Relationship between percent glycosylation of hemoglobin with fructosamine in type 2 diabetes without chronic kidney disease

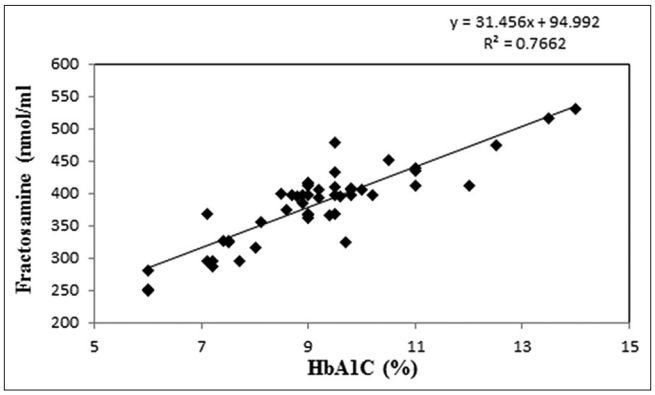

Figure 3: Relationship between percent glycosylation of hemoglobin with fructosamine in patients with type 2 diabetes with chronic kidney disease

diabetic nephropathy.[9] Other clinical reports also supported that GG was found to be the better indicator than HbA1c for assessing the progression of kidney dysfunction.[10,19,20] DCCT showed that the glycemic control as established by glycosylated hemoglobin determination is linked closely to the diabetic secondary chronic complications of CKD, retinopathy, neuropathy, and others.[21,22] Our study shows a positive linear correlation between HbA1c and FA suggesting

Table 3: Correlation analysis between different variables and glycosylation gap in patients with diabetes with chronic kidney disease

Independent variables	HbA1c (%)	Fructosamine (nmol/mg)	Glycosylation gap
Serum creatinine (mg/dl)			
R	0.0686	0.0493	−0.0511
P	0.634	0.733	0.724
eGFR			
R	−0.0481	−0.0139	0.0478
P	0.739	0.923	0.741
HDL (mg/dl)			
R	0.350	0.306	0.0687
P	0.0128	0.0307	0.634
LDL (mg/dl)			
R	0.245	0.249	0.0967
P	0.0862	0.0812	0.503
Triglycerides (mg/dl)			
R	0.281	0.257	0.145
P	0.0486	0.0719	0.314
Tricholesterol (mg/dl)			
R	0.338	0.319	0.175
P	0.0165	0.0240	0.222

Data are *r* and *P* value. eGFR: Estimated glomerular filtration rate, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, HbA1c: Glycated hemoglobin

that FA is also a good marker for monitoring the glycemic control and has important role in the onset of diabetic CKD by altering the conformation in the structure and recognized as a foreign particles by immune system of the body. MacDonald *et al*. also revealed a linear regression between HbA1c and FA levels.[23] FA values importantly increased in diabetic CKD when compared to the patients with diabetes without complications.[24] Our result shows better relation between FA and GG in CKD group. It means that GG may be the powerful clinical tool to predict the progression of diabetic CKD. The study demonstrated that a significant elevation in the serum glucose and MBG in both patients with type 2 diabetes with and without CKD group as compared to the control group that may be associated with the intravascular hyperglycemia.

The significant elevation of fructosamine level in patients with CKD can be related to increase the process of nonenzymatic glycation under the hyperglycemic condition. The products of nonenzymatic glycation (amadori as well as AGEs) accumulate in kidney tissues and play an important role in diabetic nephropathy. The high level of HbA1c in patients with type 2 diabetes with or without CKD can also be related to sustained hyperglycemia that occurs due to the impaired glucose metabolism. The substantial increase in GG in CKD group may be related to the significant elevation of measured HbA1c value, obtained from the regression equation using serum FA. The study also revealed that predicted HbA1c also increases with the progression of chronic complications as compared to patients with diabetes

without complication. This may be due to the increase in extracellular (FA) and intracellular HbA1c glycation. The study concluded a significant intracellular glycosylation process which may be the underlying cause of diabetes and its associated chronic complications. Further studies are required to investigate the exact role of GG in diabetes and related complications.

In summary, we observed consistent discordances between HbA1c and an extra-erythrocyte measure of glycemic control, FA. When this discordance is quantitated in the form of a GG, it correlates with the frequency of a major microvascular complication of diabetes.

Conclusion

FA together with GG may be considered a correct interpretation of the glycosylation processes and GG and will be useful as a research probe quantitating variation between intracellular and extracellular glycemic control to identify sources of population variation in diabetic complications beyond glycemic control.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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