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Levels of glycated albumin at different stages of diabetic nephropathy in India

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Abstract
Aims: Glycated haemoglobin (HbA1c) which is an index of long term glycaemic control in diabetic patients is measured in majority of patients worldwide. Glycated albumin (GA) is useful for the evaluation of short term glycaemic control (2 weeks) in patients with diabetes. The aim of this study was to assess the GA levels at different stages of diabetic nephropathy in Indian population. **Materials and Methods:** A total of 147 subjects (M:F; 95:52) were selected for this study and were divided into three groups based on their renal function and compared with a non diabetic control group (n = 50, M:F; 14:36). The groups were as follows; group1 (control) n = 50, group2 (normoalbuminuria) n = 42, group 3 (microalbuminuria) n = 55, group 4 (proteinuria) n = 50. GA was measured by enzymatic procedure using the Lucica GA – L kit (Asahi Kasei Pharma Corp, Japan). **Results:** The normal cutoff value for GA was derived using control group and it was found to be 15% (range 7-17%). GA was significantly higher in diabetic patients at different stages of diabetic nephropathy compared to non diabetic control group [cont: 12.9 ± 1.8, normo: 20.8 ± 5.8, micro: 26.1 ± 8.6, macro: 23.5 ± 8.3). Microalbuminuric patients had significantly higher GA levels than normoalbuminuric patients ($p< 0.05$). Proteinuric subjects had slightly lower GA levels compared to microalbuminuric group but it was not statistically significant. **Conclusion:** GA was found to be a better marker for evaluating short term glycaemic status among diabetic patients with different degree of renal impairment prior to ESRD.

Keywords: *Glycated albumin, diabetic nephropathy, type 2 diabetes, India.*

Introduction

The concept of estimating glycated albumin (GA) is proving to be an important tool in assessing short term glycaemic control of diabetic patients. Previously adequate studies have been done to prove the efficacy of HbA1c, but considering its limitations, certain other tests like glycated proteins is being looked at to overcome such circumstances. Glycated Haemoglobin (HbA1c) which is an index of long term glycaemic control (2-3 months) in diabetic patients is measured in majority of patients worldwide.[1-3] Since glycation takes place throughout the life span of hemoglobin and serum proteins, glycated albumin has been thought to reflect short term variations of glycaemic control. Levels of glycated protein reflect the degree of hyperglycemia during their life span. The turnover of serum albumin is more rapid (15-20 days) than haemoglobin, hence glycated albumin is useful for the evaluation of short term glycemic control (2 - 4 weeks) in patients with diabetes.[4-7]

Glycated proteins influence renal function and thus alter the permeability properties of the glomerular capillary wall[8] and are preferentially transported across the glomerular filtration barrier into the mesangial space.[9] Earlier work suggests preferential tubule cell uptake of the glycated forms of protein which have adverse effect on renal microvasculature. One report suggested that GA was low in end stage renal disease (ESRD) patients with nephrosis or on peritoneal dialysis since albumin leaks into the peritoneal dialysis solution; and as a result GA is not a good indicator for blood glucose control among these patients.[10] Inaba *et.al* suggested that albumin turnover should change in patients with chronic renal failure having massive proteinuria, in whom GA levels theoretically should be reduced as a result of shorter exposure to plasma albumin.[11]

There are no reports available on levels of glycated albumin at different stages of diabetic nephropathy prior to ESRD from Indian population. Therefore, this study was planned with the objective of determining the levels of glycated albumin at different stages of renal impairment prior to ESRD stage in diabetic subjects.

Materials and Methods

Subjects

A total of 147 (M: F; 95:52) type 2 diabetic subjects were selected for this cross sectional study from the outpatient department of a tertiary care centre for diabetes in India and were compared with 50 (M: F; 14:36) non diabetic control subjects. The control subjects were the attenders of the patients who had participated in the study. After the full explanation of the study, written informed consent was

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obtained from each study subject. The study was approved by the Ethics committee of the institution prior the informed consent was obtained from all the subjects.

Demographic and anthropometric details like age, weight, height, duration of diabetes, duration of diabetic nephropathy were recorded for all the study subjects. Family history of diabetes and hypertension, smoking and alcohol consumption habits were obtained from the medical records of the study subjects. Body Mass Index (BMI) (kg/m^2) was calculated using the standard formula. Blood pressure was measured in all the study subjects using a standard mercury sphygmomanometer. Blood samples were collected for the biochemical estimations. Fasting and post prandial samples were collected from the known cases of diabetes and other subjects underwent a standard oral glucose tolerance test. The diagnosis of diabetes was based on previous history of diabetes or on the criteria of World Health Organization for the classification of glucose tolerance.[12]

Type 2 diabetic subjects were divided into 3 study groups based on their renal status. Group 1 consisted of non diabetic control subjects (n = 50, M: F; 14:36). The study groups were as follows: Group 2 (n = 42, M: F; 25:17) were the normoalbuminuric subjects having random urinary albumin by creatinine ratio (ACR) of <30ug/mg creatinine estimated by using immunoturbidimetric method. Group 3 (n = 55; M: F; 36:19) were the microalbuminuric subjects having urinary albumin by creatinine ratio of 30 - 300ug/mg creatinine, group 4 (n = 50; M: F; 34:16) were the macroalbuminuric subjects having massive proteinuria of expected protein excretion rate of >500mg/day with the presence of diabetic retinopathy.

GA and HbA1c estimations

Plasma glycated albumin (GA) levels were measured by an enzymatic method using albumin specific protease, ketoamine oxidase and albumin assay reagent on the Hitachi autoanalyser 912 (Lucica GA-L, Asahi Kasei Pharma Corp, Tokyo, Japan).[13,14] GA was hydrolyzed to amino acids by albumin specific proteinase and then oxidized by ketoamine oxidase to produce hydrogen peroxide, which was measured quantitatively. The GA value was calculated as the percentage of GA relative to total albumin, which was measured with bromocresol purple method. The measured values of GA was not influenced by the substances such as bilirubin F up to 14.6 mg/dl, bilirubin C up to 15.2 mg/dl, glucose up to 1000 mg/dl, ascorbic acid up to 100 mg/dl. Glycosylated haemoglobin (HbA1c) was estimated by the turbidimetric inhibition immunoassay using haemolyzed whole blood on the Hitachi autoanalyser 912.

Other biochemical estimations

All the biochemical estimations were done by using standard enzymatic procedures. Plasma glucose was estimated by glucose oxidase peroxidase method. Renal parameter like urea was estimated by kinetic enzymatic UV assay, serum creatinine was estimated by Jaffe's kinetic method and serum albumin was estimated by bromocresol green method. Glomerular filtration rate (eGFR) was calculated using Cockroft Gault formula.[15]

Statistical Analysis

All statistical analyses were performed using SPSS 10.0 Version software (SPSS Inc, Illinois). Mean and standard deviation for continuous variables and percentages for categorical variables are reported as relevant. Significant differences between groups were evaluated using the *t* test, Chi-square test and ANOVA where ever appropriate. A p value of <0.05 was considered statistically significant.

Results

The normal cut off value for GA was derived using control group (mean+1SD). The cut off value for GA (rounded off) in this population was 15% (Range 7-17%). Table 1 shows the demographic, anthropometric and hemodynamic details of the study groups. The control subjects are younger than the study subjects. There was no significant difference between the study groups with respect to age and duration of diabetes. Body Mass Index (BMI) was lower in group 1 when compared with other study groups. The BMI of group 4 subjects was slightly higher than groups 2 and 3. No significant difference was noted in the blood pressure values between the study groups. Prevalence of hypertension increased with deteriorating renal status.

Table 2 shows the biochemical details of the study groups. Plasma glucose values and HbA1c % was higher in study groups compared with group 1. The levels of GA was significantly higher in diabetic patients at different stages of diabetic nephropathy compared to non diabetic control group [Group 1, mean ± SD: 12.9 ± 1.8; Group 2, 20.8 ± 5.8; Group 3, 26.1 ± 8.6; Group 4, 23.5 ± 8.3, respectively]. Microalbuminuric subjects (Group 3) had significantly higher GA levels than normoalbuminuric group (Group 2) ($p<0.05$). Proteinuric subjects (Group 4) had slightly lower GA levels compared to microalbuminuric subjects (Group 3) but it did not reach statistical significance. As expected, the renal parameters are elevated in the proteinuric subjects when compared with the subjects in the early stages of diabetic nephropathy.

Table 3 shows the characteristics of the study subjects stage wise as per KDOQI guidelines. A large number of subjects had positive family history of diabetes. The prevalence of hypertension, smoking and alcohol habits increased with deteriorating renal status. The levels of GA% were significantly higher in subjects with eGFR 60-89 ml/min than in subjects with eGFR ≥90ml/min. The GA levels were slightly lower in subjects with eGFR 30-59ml/min than eGFR 60-80ml/min.

Discussion

To our knowledge this is the first cross sectional study from India that assessed the levels of GA at different stages of diabetic nephropathy among type 2 diabetic patients. Based on the present study findings, it was found that GA is a better indicator for evaluating short term glycemic control in type 2 diabetic patients with different degrees of renal

Table 1: Demographic, anthropometric and haemodynamic details of the study groups.

Variable	Group 1 n = 50	Group 2 n = 42	Group 3 n =55	Group 4 n =50	p value ANOVA between study groups
M:F	14:36	25:17	36:19	34:16	
Values are mean ± SD					
Age (years)	34.0 ± 7.8	49.0 ± 6.1	47.0 ± 6.6	48.0 ± 6.4	0.312
BMI (kg/m^2)	24.3 ± 3.2	28.7 ± 5.7	28.5 ± 4.8	31.0 ± 5.8	0.040
SBP (mmHg)	119.4 ± 4.6	120.7 ± 7.1	118.4 ± 7.8	120.8 ± 11.7	0.323
DBP (mmHg)	80.2 ± 1.4	81.0 ± 5.8	80.9 ± 6.7	81.4 ± 6.4	0.916
Duration of DM (years)	_	7.3 ± 3.8	9.2 ± 5.5	9.0 ± 4.2	0.104
Values are n (%)					
HTN (%)	1 (2.0)	3 (7.1)	17 (30.9)	46 (92.0)	<0.0001
FH of DM (%)	_	14 (33.3)	38 (69.1)	28 (56.0)	0.002
FH of HTN (%)	_	4 (9.5)	15 (27.3)	8 (16.0)	0.071
Smoking (%)	_	1 (2.4)	10 (18.2)	6 (12.0)	0.054
Alcohol (%)	_	1 (2.4)	3 (5.5)	4 (8.0)	0.496

SBP= Systolic blood pressure, DBP=Diastolic blood pressure, DM=diabetes, HTN=Hypertension, FH=Family history

Table 2: Biochemical details of the study groups

Variable	Group 1 n = 50	Group 2 n = 42	Group 3 n =55	Group 4 n =50
Plasma Glucose (mmol/L) Fasting	4.9 ± 0.38	8.6 ± 3.15*	9.9 ± 3.72*	11.3 ±5.03*,**
Plasma Glucose (mmol/L) 2 hr	5.4± 0.75	13.1 ± 4.08*	15.4 ± 5.38*	15.3 ± 5.70*
S. Albumin (g/L)	41 ± 3.9	39 ± 6.9	37 ± 9.6	36 ± 9.9
HbA1c (%)	5.6 ± 0.27	8.4 ± 2.1*	10.4 ± 2.4*,**	9.95 ± 2.4*,**
GA (%)	12.9 ± 1.8	20.8 ± 5.8*	26.1 ± 8.6*,**	23.5 ± 8.3*
Urea (mmol/L)	--	7.9 ± 2.71	8.3 ± 2.32	13 ± 5.07**,#
Crea (µmol/L)	--	61.8 ± 21.2	72.4 ± 21.2	114.9 ± 65.5**,#

Values are mean ± SD P<0.05* Vs group 1; ** Vs group 2;# Vs group 3

Table 3: Clinical characteristics of the study subjects stage wise as per KDOQI guidelines

Variable	eGFR ≥90ml/min n = 94	eGFR 60–89 ml/min n = 37	eGFR 30–59ml/min n =16	p value ANOVA between groups
M:F	57: 37	28 : 9	10 : 6	
Age (years)	47.5 ± 6.5	48.7 ± 5.7	47.6 ± 7.6	0.625
Values are n (%)				
FH of DM	51 (54.3)	18 (48.6)	11 (68.8)	0.402
HTN	35 (37.2)	16 (43.2)	15 (93.8)	<0.0001
Smoking	9 (9.6)	5 (13.5)	3 (18.8)	0.502
Alcohol	3 (3.2)	3 (8.1)	2 (12.5)	0.225
Values are mean ± SD				
HbA1c (%)	9.9 ± 2.5	9.6 ± 2.4	8.0 ± 1.5*	0.011
Urea (mmol/L)	8.0 ± 2.0	11 ± 4.4*	18.2 ± 5.6*,**	<0.0001
Crea (µmol/L)	60.9 ± 12.4	97.2 ± 21.2*	185.6±68.9*,**	<0.0001
GA (%)	22.6 ± 7.0	27.1 ± 9.3*	22.2 ± 8.7	0.012

* Vs ≥ 90ml/min ** Vs 60-89 ml/min

impairment prior to ESRD. Many studies have examined and shown the significance of determining GA than HbA1c in the diabetic patients on hemodialysis.[11,16] Earlier studies among diabetic subjects on hemodialysis confirmed that HbA1c levels significantly under estimate glycemic control and GA may be a better indicator to assess glycemic control in dialysis patients with diabetes. However, there exists a limitation of assessing GA levels in patients with massive proteinuria .[11] The GA levels may be reduced in patients with proteinuria because of shorter exposure to plasma albumin or due to a change in albumin turn over. The present study attempted to see the GA levels at different stages of diabetic nephropathy prior to ESRD. The levels of GA in type 2 diabetic patients were higher than non diabetic control subjects. The normal cut off value for GA in our population was derived using control group and it was 15% (range 7-17%). Our cut off value was within the range reported in the Japanese population. The reference interval

of GA was 12.3 – 16.9% according to Japan Diabetes Society. The normal GA % range in US population (11.6% ± 1.6) was slightly lower than our cut off value.[16] The slight differences might be noted because of the use of different assay methodology. Further studies are required to confirm the target GA levels that are necessary to ensure a good prognosis for patients with diabetes.

In the present study, the GA levels are slightly low in the proteinuric subjects but it did not reach the statistical significance and the values were comparable among the groups. Similar results were observed when the subjects were divided into groups as per KDOQI guidelines. It was reported that the GA levels are low in ESRD patients probably because serum albumin levels were low as a result of nephrosis. It was shown that GA is not a good indicator for blood glucose control among these patients. The authors reported that if the serum albumin levels are above 3.5g/dl, the GA levels of ESRD patients are comparable to the GA levels of patients with normal renal function.[10] In the present study subjects, albumin levels were >3.5g/dl at different stages of renal impairment. It could be the reason that the GA levels are comparable among these subjects also. Another reason may be the use of improved method of GA estimation, which is free of interference by endogenous glycated amino acids and is unaffected by changes in albumin concentration.[14]

More studies are needed in this population to determine until which stage of diabetic kidney disease can measurement of GA becomes preferable. There is a need to plan further studies to determine the efficacy or usefulness of GA in comparison with HbA1c at different stages of diabetic kidney disease. GA increases prior to HbA1c in patients with deteriorating glycemic control, so we need to study and confirm the clinical significance of GA in diabetic complications. In conclusion, GA can be used as an indicator for assessing short term glycemic control in type 2 diabetic subjects with different degree of renal impairment prior to ESRD.

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