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Glycated Albumin as a Prognostic Indicator of Cardiovascular Events in Patients with Diabetic Nephropathy

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***Abstract**: Introduction: Diabetic Asian Indians compared to westerners are highly prone to end-organ complications resulting in mortality. Few studies are being done in India to assess cardiac complications in diabetic nephropathy. This study was done to evaluate the role of glycated albumin in assessing cardiovascular events in diabetic nephropathy patients.*
***Materials and methods:** The study was conducted at Sri Ramachandra Medical College and Research Institute, Chennai and G.S.L.Medical College and Hospital, Rajahmundry. This case control study included 170 participants of 45 to 60 years old from both sexes. Study had four groups; group I: 45 nondiabetic apparently healthy individuals, Groups II, III and IV individuals who had type 2 diabetes mellitus for atleast five years; 45 diabetics with normoalbuminuria, 40 diabetics with microalbuminuria and 40 diabetics with macroalbuminuria in groups II to IV respectively. The study was approved by ethics committees of both the institutes. Written informed consent was obtained. HbA1c and glycated albumin were analyzed by immunoturbidometry, ion exchange chromatography and ELISA respectively. The obtained data were checked for normal distribution. Appropriate statistical tools were used; P value less than 0.05 was considered statistically significant. The statistical analyses were performed in SPSS software version 16.0.*
***Results:** There was significant increase in glycated albumin. There was a positive correlation between GA and HbA1c. The cutoff level of GA was 176.50pmol/mL.*
***Conclusion:** Glycated albumin is a good prognostic indicator of carotid artery atherosclerosis, retinopathy and postprandial hyperglycemia. Glycated albumin levels can assess the cardiovascular events in diabetic nephropathy.*

***Keywords:** diabetic nephropathy, albuminuria, glycated albumin, glycated hemoglobin, macrovascular complications of diabetes mellitus, microvascular complications of diabetes mellitus*

1. INTRODUCTION

The prevalence of type 2 diabetes mellitus (T2DM) is predicted to increase to 4.4% (366 million) by 2030.[1][2] 20-40% of diabetic nephropathy patients (DN) end up in End Stage Renal Disease (ESRD).[3] In 2009, The American Diabetic Association (ADA) introduced glycated haemoglobin (HbA1c) as the gold standard for the diagnosis of DM.[4] HbA1c reflects glycemic status during a relatively long period (past 2 -3months).[5][6] Falsely low HbA1c values are seen in red blood cell disorders like haemolytic anaemia.[7] To have an intermediary duration of glucose status indicator, glycated albumin (GA) is being proposed.

GA reflects postprandial plasma glucose and glycemic excursions.[4] This study was undertaken to evaluate the efficacy of glycated albumin in predicting the cardiovascular complications in diabetic nephropathy patients.

2. MATERIALS AND METHODS:

Study participants:
The study was conducted in the Department of Biochemistry at Sri Ramachandra Medical College and Research Institute, SRIHER, Chennai. This case control study included 170 participants who were in the age group of 45 to 60 years from both sexes. The study participants were classified into four groups; group I consisted of 45 apparently healthy nondiabetic individuals. Groups II, III and IV consisted of individuals with history of T2DM and on treatment for a minimum period of five years. Group II consisted of 45 diabetics with normoalbuminuria, group III consisted of 40 diabetics microalbuminuria and group IV consisted of 40 diabetics with macroalbuminuria. The study participants included into the study were recruited from the Department of General Medicine at G.S.L.Medical College and Hospital, Rajahmundry. Exclusion criteria included pregnant women, individuals with hypertension, alcoholics, smokers and individuals on drugs like Angiotensin Converting Enzyme Inhibitors (ACEI) or Angiotensin Receptor Blockers (ARB). The study was approved by the ethics committees of both the institutes, Sri Ramachandra Institute of Higher Education and Research, Chennai and G.S.L.Medical College and Hospital, Rajahmundry; in accordance with the declaration of Helsinki. Written informed consent was obtained from all the study participants before they were recruited into the study.

3. METHODOLOGY:

Anthropometric measurements such as height and weight were measured and body mass index (BMI) was calculated. Both waist and hip circumferences were assessed and wait to hip ratio (WHR) was calculated. Plasma glucose and lipid profile were measured by standard methods. HbA1c was measured by Ion exchange chromatography. eGFR was calculated using MDRD equation: $1.86x\ (creatinine/88.4)^{-1.154}\ x\ (Age)^{-0.203}\ x(0.742$ if female). Urinary albumin concentration was measured using the immunoturbidometric assay. Serum glycated albumin was analyzed by ELISA (Cat, No.E0029Hu Bioassay technology laboratory).

4. STATISTICS:

The data obtained were subjected to Shapiro-Wilks test for normality. The data of all variables were found to follow normal distribution; hence expressed as mean and standard deviation. Comparison of data was done by one way ANOVA; Tukey's HSD post-hoc test was applied to assess the difference between the groups. Correlation studies were done. *P* value of less than 0.05 was considered statistically significant. Receiver Operating Characteristics (ROC) curve was done to arrive at the cutoff levels of glycated hemoglobin and glycated albumin. The statistical analyses were performed in SPSS software version 16.0.

5. RESULTS:

Table 1: Anthropometric and biochemical parameters of the study participants:

Parameters	Group I Healthy controls N= 45	Group II Diabetic normoalbuminuria N=45	Group III Diabetic microalbuminuria N=40	Group IV Diabetic macroalbuminuria N=40	*P* Value
Age in years	49.57±6.08	50.9±5.59	52.82±6.64	53.7±6.00	0.009**
DM duration (years)	0	8.13±2.17	8.85±2.17	8.80±1.68	0.0001***
BMI (kg/mt^2)	24.73±1.57	27.28±2.50	27.73±2.70	31.31±2.08	0.0001***
WHR	0.91±0.03	0.97±0.04	0.96±0.03	1.05±0.05	0.0001***
eGFR (ml/mt)	107.56±14.73	86.62±20.83	75.60±12.69	31.33±7.12	0.001***
FPG (mmol/L)	4.20±0.50	7.41±1.76	6.87±1.21	8.72±1.16	0.001***
PPPG (mmol/L)	6.30±0.74	10.87±2.55	10.06±1.70	12.32±2.08	0.001***
TC(mmol/L)	4.55±0.62	4.75±0.89	4.52±0.91	5.22±0.73	0.0001***
TGL(mmol/L)	1.51±0.21	1.92±0.63	2.03±0.71	2.23±0.52	0.0001***
HDLc(mmol/L)	1.08±0.10	1.05±0.09	1.00±0.07	0.96±0.09	0.0001***
LDLc(mmol/L)	4.40±0.77	2.84±0.76	2.57±0.81	3.23±0.65	0.0001***
HbA1C %	5.15±0.34	6.98±0.70	7.02±0.65	8.45±0.76	0.0001***
GA (pmol/mL)	97.33±21.04	194.40±59.97	211.45±76.20	379.57±133.8	0.00001****

(P value:**→highly significant, ***→very highly significant) DM: diabetes mellitus, BMI:Body Mass Index, eGFR: estimated glomerular filtration rate, FPG: fasting plasma glucose, PPPG:postprandial plasma glucose, TC: total cholesterol, TGL: triglyceride, HDLc: high density lipoprotein cholesterol, LDLc: low density lipoprotein cholesterol, HbA1c: glycated hemoglobin, GA: glycated albumin)

There was statistical significant difference in the lipid profile across the groups. HbA1c steadily increased from groups I to IV. But the magnitude of increase in GA was much more than that of HbA1c.

Table 2: Tukey's HSD post hoc test to compare between the groups

Parameter	I Vs II	I Vs III	I Vs IV	II Vs III	II Vs IV	III Vs IV
Age	0.705	0.071	0.011*	0.492	0.165	0.918
DM duration	0.0001**	0.0001**	0.0001**	0.376	0.441	1.000
BMI	0.0001**	0.0001**	0.0001**	0. 804	0.0001**	0.0001**
WHR	0.0001**	0.0001**	0.0001**	0.976	0.0001**	0.0001**
eGFR	0.001**	0.001**	0.001**	0.005	0.001**	0.001**
FPG	0.001**	0.001**	0.001**	0.196	0.001**	0.001**
PPPG	0.001**	0.001**	0.001**	0.209	0.003**	0.001**
TC	0.613	0.999	0.001**	0.555	0.038*	0.001**
TGL	0.004**	0.0001**	0.0001**	0.775	0.051	0.391
HDLc	0.515	0.001**	0.0001**	0.073	0.000**	0.247
LDLc	0.0001**	0.0001**	0.0001**	0.385	0.079	0.001**
HbA1c	0.0001**	0.0001**	0.0001**	0.994	0.0001**	0.0001**
GA	0.0001**	0.0001**	0.0001**	0.7707	0.0001**	0.0001**

(P value:**→highly significant, ***→very highly significant) DM: diabetes mellitus, BMI:Body Mass Index, eGFR: estimated glomerular filtration rate, FPG: fasting plasma glucose, PPPG:postprandial plasma glucose, TC: total cholesterol, TGL: triglyceride, HDLc: high density lipoprotein cholesterol, LDLc: low density lipoprotein cholesterol, HbA1c: glycated hemoglobin, GA: glycated albumin)

There was highly significant difference in anthropometric measurements. There were significant alterations in lipid parameters as shown by dyslipidemia. Glycemic status markers were significantly increased as the renal damage advanced.

Table 3: Correlation studies

Variables	Age	DM Duration	BMI	WHR	eGFR	FPG	PPPG	TC	TGL	HDLc	LDLc	HbA1c	GA
Age	1	0.307 0.000	0.086 0.265	0.246 0.001	-0.220 0.004	0.107 0.166	0.136 0.077	0.046 0.551	0.033 0.668	-0.215 0.005	0.122 0.112	0.135 0.079	0.134 0.082
DM duration		1	0.541 0.000	0.514 0.000	-0.548 0.000	0.635 0.000	0.594 0.000	0.097 0.209	0.392 0.000	-0.291 0.000	0.066 0.392	0.668 0.000	0.480 0.000
BMI			1	0.550 0.000	-0.616 0.000	0.539 0.000	0.513 0.000	0.295 0.000	0.335 0.000	-0.282 0.000	0.658 0.000	0.640 0.000	0.624 0.000
WHR				1	-0.680 0.000	0.595 0.000	0.593 0.000	0.289 0.000	0.316 0.000	-0.333 0.000	0.330 0.000	0.672 0.000	0.644 0.000
eGFR					1	-0.683 0.000	-0.644 0.000	0.259 0.001	0.188 0.014	-0.367 0.000	0.241 0.002	-0.770 0.000	-0.718 0.000
FPG						1	0.858 0.000	0.256 0.001	0.390 0.000	-0.336 0.000	0.261 0.392	0.880 0.000	0.758 0.000
PPPG							1	0.233 0.002	0.401 0.000	-0.334 0.000	0.214 0.005	0.795 0.000	0.630 0.000
TC								1	0.323 0.000	-0.078 0.315	0.845 0.000	0.253 0.001	0.239 0.002
TGL									1	-0.075 0.329	0.204 0.008	0.424 0.000	0.349 0.000
HDLc										1	0.036 0.639	-0.407 0.000	-0.364 0.000
LDLc											1	0.245 0.001	0.254 0.001
HbA1c												1	0.906 0.000
GA													1

(P value:**→highly significant, ***→very highly significant) DM: diabetes mellitus, BMI:Body Mass Index, eGFR: estimated glomerular filtration rate, FPG: fasting plasma glucose, PPPG:postprandial plasma glucose, TC: total cholesterol, TGL: triglyceride, HDLc: high density lipoprotein cholesterol, LDLc: low density lipoprotein cholesterol, HbA1c: glycated hemoglobin, GA: glycated albumin)

In this study there was a positive correlation between HbA1c and GA (r = 0.906, *P*= 0.000) among all groups.

Figure 1: Receiver operating Curve characteristics done to arrive at the cut off levels of HbA1c and GA

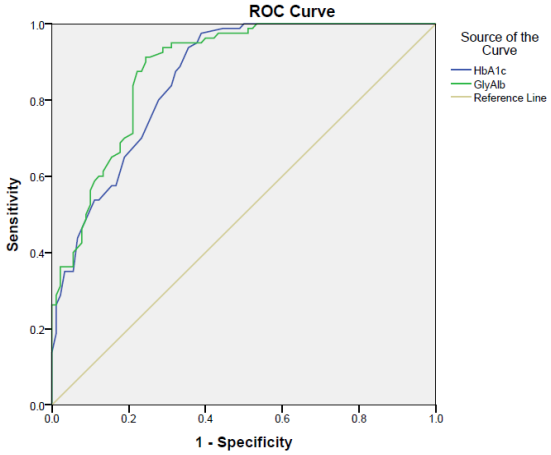


Table 4: Cutoff levels and area under the curve for HbA1c and GA

Variables	Cutoff level	Sensitivity %	Specificity %	AUC
HbA1c%	6.55	88	67	0.858
GA(pmol/mL)	176.50	90	76	0.882

(HbA1c: glycated hemoglobin, GA: glycated albumin, AUC: Area Under the Curve)

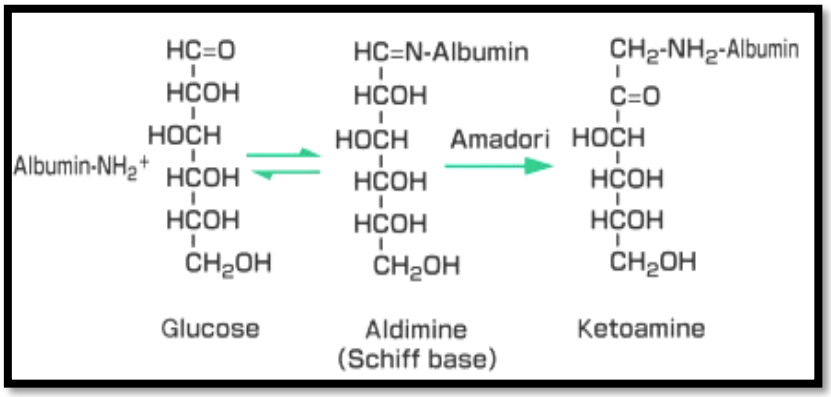
The cutoff level of HbA1c to identify cases of diabetic nephropathy with cardiac complications is 6.55% with sensitivity of 88% and specificity of 67%. The cutoff level of GA is 176.50pmol/mL with sensitivity of 90% and specificity of 76%.

6. DISCUSSION:

In Southeast Asia, the prevalence of T2DM was 82 million in 2017 which might double to 151 million in 2045.[8] As per Diabetes Atlas 2006, India has the largest number of T2DM patients in the world, estimated tobe 40.9 millions in 2007 which could increase to 69.9 million in 2025. T2DM in Indians differs from that of Westerners with respect to the younger age of onset, not all cases are associated with obesity and the predominant presence of genetic factors.[9]

Longstanding hyperglycemia causes glycation of proteins; glycation is a non-enzymatic process of attaching glucose to certain specific amino acids in proteins.[10] The extent of glycation depends on the protein characteristics such as: location (extracellular or intracellular), half life, concentration and amino acid composition of the protein in addition to age, race and ethnicity of the individual. Hemoglobin present in erythrocytes forms HbA1c; its measurement gives the data about the glucose status of the individual in the past 2 to 3 months. The Diabetes Control and Complications Trial (DCCT) and U.K. Prospective Diabetes Study (UKPDS) have demonstrated that the progression of diabetic microangiopathy is correlated with HbA1c.[4] Glycation occurs in two stages; in the first stage glucose gets attached to the amino groups of amino acids such as lysine or arginine on proteins to form a Schiff base. In the second stage, the Schiff base undergoes a rearrangement to create a stable Amadori product, or ketoamine. (Figure 2) Further oxidation, dehydration, and cross-linking steps then occur forming reactive dicarbonyl compounds called advanced glycated end products (AGEs).[10]

Figure 2: shows the steps of formation of glycated proteins [11]



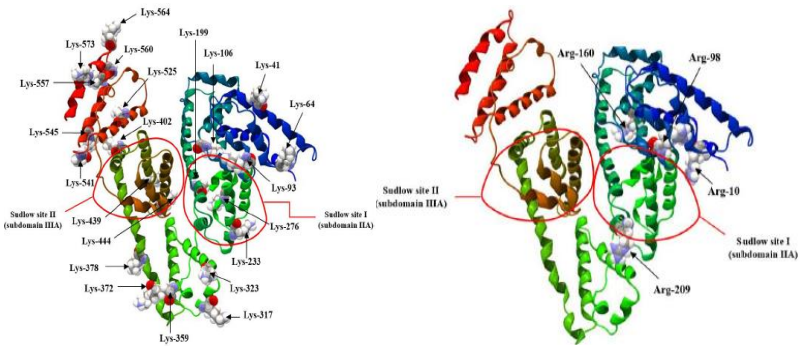
As per ADA guidelines, application of HbA1c in glucose monitoring becomes questionable in patients receiving hemodialysis (HD), to assess postprandial hyperglycemia, hypoglycaemia and unexpected oscillations in glucose levels in blood.[12] HbA1c does not

reflect the actual glucose status in certain conditions. The conditions in which HbA1c values are falsely high include iron deficiency anemia, pregnancy and variant hemoglobin. The conditions in which HbA1c levels can be falsely low include diseases with shortened lifespan of RBCs, hemolytic anemia, liver cirrhosis, chronic kidney disease, during treatment of iron deficiency anemia, variant hemoglobin, neonates, and hereditary persistence of fetal hemoglobin (HPFH).[4] There could be inter-individual heterogeneity in HbA1c due to transfer of glucose across RBC membranes.[13] In these situations it is better to have a marker which might be stable in the above mentioned conditions as well as reflect glucose level in a shorter duration. Glycated albumin (GA) is found to be appropriate in the above mentioned conditions.

Fructosamine (FA) refers to all glycated serum proteins with ketoamine linkages.[10] FA consists of glycation of albumin (around 90%), apolipoprotein B100 present on LDL, IgA, collagen and laminin. GA is considered to be a glucose status indicator for 1 to 3 weeks since the half life of albumin is 20 days. Hence GA gives a data of shorter duration changes in glycemic control, hemolytic anemia, diabetic retinopathy, T2DM with comorbidities or who is on HD.[14] GA was associated with intima media thickness (IMT) of carotid arteries, dementia and cancer.[12] GA values are also correlated with postprandial plasma glucose levels and oscillations in plasma glucose concentrations.[12][13][15] Albumin is highly sensitive to glycation; it is 4.5 times faster than HbA1c, probably due to two reasons: albumin is present extracellularly and has more glycation sites. GA is present at a concentration of 1% to 10% in normal individuals.[12][13] It increases to two to three fold in T2DM.[10] In chronic inflammation, smoking, hyperuricemia, hypertriglyceridemia, non-alcoholic fatty liver disease (NAFLD), nephrotic syndrome, hyperthyroidism, administration of glucocorticoids, Cushing's syndrome and neonates/infants, apparently low GA levels were observed. On the other hand, in liver cirrhosis and hypothyroidism, apparently high GA levels were observed.[4]

In the present study, HbA1c was 5.15±0.34, 6.98±0.70, 7.02±0.65 and 8.45±0.76 in groups I to IV respectively. There was highly significant difference between the groups as shown by $P = 0.0001$.(Table 1) As the kidney function deteriorated, there was found to significant increase in the HbA1c values.(Table 2) Various articles have expressed reference values for GA in %. In the present study, GA was expressed in pmol/mL units, GA as % was not estimated. The reference interval of GA as mentioned in the kit insert for the methodology was 100-285pmol/mL; the same had been followed in this study as well. GA was normal in group I, but had increased further from groups II to IV corresponding to the deterioration in kidney function.(Table I). There was significant difference between the groups in HbA1c and GA; but this significant difference did not exist between groups II and III. This was probably due to the fact that the extent of damage was almost the same in both stages of normoalbuminuria and microalbuminuria. (Table 2) When HbA1c was compared with GA there was significant correlation with $r = 0.906$, $P = 0.000$. (Table 3)

Figure 3 (a & b) shows the location of lysine and arginine amino acids in human serum albumin which are the sites of glycation[13]



Albumin has a molecular weight of 66.7 kDa and it increases by 180 daltons with each glucose molecule being added. It has a single polypeptide chain with 585 amino acids; glucose gets attached to lysine at positions 525, 439, 199, 51, 378, 545, 12, 233, 276, 281, 317, 323, N-terminus and another 23 lysine residues; with 24 arginine residues in addition.[16] This causes conformational change in structure of albumin which gets reflected as alteration in the function of albumin also. Albumin has lot of physiological functions such as regulation of osmotic pressure and blood pH, role as an antioxidant and a carrier of various compounds. Till date lot of research has been done to elucidate the structural alterations of albumin in diabetic patients but functional alterations have not been studied.[10] Instead of using HbA1c or GA as glycemic status indicators, GA:HbA1c ratio is a better indicator, because it takes into false variations of both variables.[4]

Diabetic patients with features of metabolic syndrome (MetS) and insulin resistance (IR) have twice the risk of death from cardiovascular disease (CVD) compared to those without microalbuminuria.[17] The adverse progression of microalbuminuria is due to the formation of AGEs, increased oxidative stress, activation of renin angiotensin aldosterone system (RAAS), activation of inflammation, and fibrosis pathways in addition to genetic and environmental factors.[17] In the Heart Protection Study, 40 mg simvastatin was found to reduce major vascular events, independent of cholesterol levels at baseline.[18] Obesity alone has been associated with glomerular hypertrophy, increased urinary albumin excretion (UAE), and even decreased glomerular filtration rate (GFR) in the absence of T2DM.[19]

This study was conducted as a case control study, the mean and SD of group I was 49.57±6.08 years. Whereas the mean ages were 50.9±5.59, 52.82±6.64 and 53.7±6.00 in groups II to IV respectively. (Table 1) In Tukey's HSD post hoc analysis it was found that there was significant increase only when group I was compared with group IV; probably it indicated that advanced renal disease with eGFR of 31.33±7.12ml/mt was found in the advanced age group. Otherwise there was no significant difference between other groups. (Table 2) In the present study correlation analysis showed that there was positive correlation of age with DM duration and WHR, and negative correlation with eGFR and HDLc. There was positive correlation of DM duration with BMI, WHR, fasting plasma glucose (FPG), postprandial plasma glucose (PPPG), TGL, HbA1c and GA; and negative correlation with eGFR and HDLc. (Table 3)

Table 5: BMI classification as per WHO criteria and Asia Pacific Guidelines[20]

BMI (Weight in Kg /Height in meters^2)	WHO criteria	Asia Pacific Guidelines
Underweight	<18.5	<18.5
Normal	≥ 18.5 but <25	≥18.5 but <23
Overweight	≥ 25 but <30	≥23 but <25
Obesity	>30	≥ 25
Obesity Grade I	≥ 30 but <35	≥ 25 but <30
Obesity Grade II	>35	>30

In the present study, the study participants were in the overweight category according to Asia Pacific Guidelines, with BMI of 24.73±1.57, whereas the diabetic patients from groups II to IV were in the obese category with BMI of 27.28±2.50, 27.73±2.70 and 31.31±2.08 respectively which were statistically significant with $P = 0.0001$. (Tables 1 & 2) There was significant difference in waist: hip ratio (WHR) in groups I to IV with values of 0.91±0.03, 0.97±0.04, 0.96±0.03 and 1.05±0.05 respectively. As per WHO guidelines, a BMI of more than 30 kg/m2 indicates general obesity; waist circumference more than 94 cm in males and 80 cm in females indicate central/abdominal or upper body obesity.[21] The healthy BMI for an urban Indian is less than 23 kg/m^2, and waist circumference should be less than 85 cm in men and 80 cm in women, and WHR of less than 0.89 for men and 0.81 for women.[22] Obesity, either generalized or abdominal, is found a play a crucial role in the pathophysiology of DN. There is glomerular hyperfiltration, increased renal tubular sodium reabsorption, impairment of renal autoregulation and increase in systemic blood pressure.[21] In the present study there was significant difference in BMI as well as WHR between the groups. (Table 2) Obesity being associated with DN is found in more than 70% of the diabetic population.[23] It was found that there was 12% reduction in the levels of serum creatinine and cystatin C after weight reduction.[17] The influence of BMI on DN progression was investigated retrospectively in 125 hospital-based cohorts over a mean follow-up period of approximately 10 years. They found that BMI, at presentation was strongly and independently associated with chronic kidney disease progression.[24]

In the present study anthropometric parameters and plasma glucose levels were correlated with other variables. There were positive correlations of BMI with WHR, plasma glucose, TC, TGL, LDL, HbA1c and GA and negative correlations with eGFR and HDLc. When WHR was correlated with other variables, there were positive correlations with plasma glucose, TC, TGL, LDLc, HbA1c and GA; and negative correlations with eGFR, HDLc. eGFR showed positive correlations with TC and LDLc and negative correlations with FPG, PPPG, HDLc, HbA1c and GA. Fasting and postprandial plasma glucose levels showed positive correlations with TC, LDL, TGL, HbA1c and GA. (Table 3)

IR present in T2DM leads to release of free fatty acids (FFA) from adipose tissue. These FFA get incorporated into VLDL in the liver and get secreted into systemic circulation causing postprandial lipemia. In these patients if there is associated increase in LDLc, CVD risk increases many folds.[25] LDL can be large in size with diameter more than 255 A˚ (pattern A) or small in size with diameter less than 255 A˚(pattern B); pattern B is associated with IR. HDLc concentrations are decreased in hypertriglyceridemic individuals due to insufficient exchange of cholesterol for TGL between HDL to TGL rich lipoproteins which is mediated by cholesterol ester transfer protein. Hyperinsulinemia causes increased fractional clearance rate of apolipoprotein A-I, a constituent of HDL leading to deceased HDLc concentration.[25] Chang et al. demonstrated that T2DM patients with high HDL had a reduced risk of DN.[17] HDLc may undergo glycation leading to dysfunctional HDLc, leading to glomerulosclerosis and tubulointerstitial damage.[26] In diabetic Japanese serum TGL levels were better predictors of CVD risk than HbA1c. In diabetics there is increased Nieman Pick Cell1Like 1(NPC1L1), reduced ATP binding cassette (ABC)G5/G8 expressions and microsomal triglyceride transfer protein (MTP) in the intestine, leading to high prevalence of hypercholesterolemia and hyperchylomicronemia.[27]

In diabetic patients, hyperglycemia causes vascular endothelial damage decreased activity of lipoprotein lipase (LPL). This results in increased TGL and decreased HDLc levels. In the early stages, DN presents with postprandial hypertriglyceridemia, whereas in the later stages there is hypercholesterolemia. Serum amylod A (SAA) is mostly carried by HDL; during HD, SAA replaces apoAI in HDL. This interferes with reverse cholesterol functions of HDLc.[28]

HDLc having antioxidant and anti-inflammatory properties modulates glucose uptake in skeletal muscle.[29] In the present study total cholesterol was higher than normal in group IV, whereas it was within the normal reference interval.(Table1) There was statistical significant difference in total cholesterol when group IV was compared with other groups I to III. (Table 2) TGL was higher than normal in the diabetic groups II to IV which were statistically significant with $P = 0.0001$(Table 1). Since TGL was elevated in groups II to III, when Tukey's HSD post hoc was done there was statistical significant difference when group I was compared with Groups II, III or IV. (Table 2) HDLc was low in groups III and IV; whereas it was low normal in groups I and II and there was statistical significant difference as shown in table 1. There were statistical significant difference in HDLc in groups III & IV when compared with groups I and II. (Table 2) LDLc was less than normal in group III. It was much higher in group I with normal total cholesterol and HDLc. Probably there was incipient hypercholesterolemia which was not noticed, since the participants were presumed to be apparently healthy individuals. In group IV LDLc was higher probably due to advancing diabetic nephropathy. When compared within the groups, LDLc in group I showed significant difference with groups II, III and IV as well as between groups III and IV. (Table 2)

In the present study total cholesterol, LDLc and TGL had positive correlations with HbA1c and GA; whereas HDL had negative correlations with HbA1c and GA. (table 3) Obesity causes excessive deposition of diacylglycerol and toxic metabolites derived from TGL in the kidneys. They cause mitochondrial dysfunction, endoplasmic reticulum stress, apoptosis, and eventually renal dysfunction.[21][30][31] Treatment of MetS was associated with a lesser progression of nephropathy in T2DM.[17]Studies have demonstrated that weight loss there was an improvement in albuminuria and dyslipidemia.[24]

In the present study as per table 4, the cutoff level of HbA1c to identify cases of diabetic nephropathy with cardiac complications was 6.55% with sensitivity of 88% and specificity of 67%. The cutoff level of GA was 176.50pmol/mL with sensitivity of 90% and specificity of 76%. The area under the curve for HbA1c and GA were 0.858 and 0.882, showing that GA is a better prognostic marker of CVD in DN with better sensitivity and specificity than HbA1c. (Figure1) The International Expert Committee, the ADA, and the WHO proposed the use of HbA1c to diagnose diabetes and pre-diabetes at a threshold of 6.5% and 5.7%, respectively. In the Japanese population, Mukai et al. reported the prevalence of diabetic retinopathy increased sharply when GA was between 16.2% and 17.5%. [12][32] The reference interval for GA varies according to the various methodologies adopted such as chromatography, enzymatic method or ELISA. Thus various studies have reported normal value of GA either as 5–9% or as 10–20%.[33]

Clinical reports in Korea and Japan have shown a wide range of GA:HbA1c ratio from 2.0 to 4.0.[14] GA:HbA1c ratio of 2.8 has been proposed by the National Glycohemoglobin Standardization Program (NGSP) in a Japanese population.[18] GA/A1c ratio increases more rapidly than A1c level which may be attributed to more marked increases in GA levels relative to A1c levels.

7. LIMITATIONS OF THE STUDY:

The sample size is small in the individual groups. The study could have been conducted as cohort study to study cause and effect relationship. It should have been possible to express glycated albumin as percentage instead of pmol/mL, which could have made calculation of the GA:HbA1c ratio feasible.

8. CONCLUSIONS:

Glycated albumin was positively associated with DN. GA interacts with receptors on mesangial cells in kidney and receptors on the macrophages of the artery walls thus activating

widespread low-grade inflammation, with loss of antioxidant activity of albumin, the net result is formation of atheroma plaques. It has been reported that the GA:HbA1c ratio is more closely associated with postprandial plasma glucose levels and insulin secretory function than HbA1c. GA:HbA1c ratio rather than GA alone has been reported to be a useful index of glucose excursion and is related to beta cell function.

9. ACKNOWLEDGMENTS:

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