Jurnal Ilmu Kedokteran (Journal of Medical Science), Jilid 17, Nomor 1, Maret 2023, Hal. 53-58
https://doi.org/10.26891/JIK.v17i1.2023. 53-58

Comparations Of Glycated Albumin Among Type 2 Diabetes Mellitus Patient With Normoalbuminuria, Microalbuminuria And Macroalbuminuria

Raja Iqbal Mulya Harahap[1*], Nina Tristina[1], Ruri Rizki Anriani[1]

ABSTRACT

Diabetic nephropathy is the most often complication of diabetes mellitus (DM). Complications of diabetic nephropathy can occur through several pathways, one of which is the formation of Advanced Glycation End Products (AGEs) through the formation of Glycated Albumin (GA). Glycated albumin (GA) is a medium-term glycemic control (2-3 weeks), shorter duration than HbA1c (2-3 months). The aim of this study was to compare the levels of GA in T2DM patients with normoalbuminuria, microalbuminuria, and macroalbuminuria. This is a cross-sectional study with subjects were 105 outpatients who had been diagnosed with T2DM by clinicians at Hasan Sadikin Hospital's Endocrinology Clinic. We examined urinary albumin per Creatinine (u-ACR) and blood GA Increased GA level was found in 75.24% subjects. Kruskal Wallis test in GA serum levels of patients with T2DM normoalbuminuria group, microalbuminuria and macroalbuminuria revealed statistically significant ($p < 0.001$), with median and range of GA in normoalbuminuria 15,9% (12,1-21,89)%; microalbuminuria 20,9% (12,9-47,2)%; dan macroalbuminuria 23,1% (13,6-46,1)%. Statistical analysis showed that the GA serum level was significantly different between T2DM patients with normoalbuminuria and macroalbuminuria, but not between those with microalbuminuria and macroalbuminuria ($p=0.001$ and $p=0.137$, respectively). The result showed that the extensive kidney damage at the subject, the higher result of Glycated Albumin level in serum.

Keywords: Glycated Albumin (GA), Type 2 Diabetes Mellitus (T2DM), urinary Albumin per Creatinine Ratio (uACR)

Diabetes mellitus is a group of non-communicable and metabolic disease that still becomes universal health problem, especially in developing country, such as Indonesia. Worldwide, almost 30 million cases in 1985 increase up to 382 million cases in 2013, and 407 cases in 2019. International Diabetes Federation (IDF) estimates the amount of Diabetes Mellitus people will rise up to 642 million people in 2040. According to *World Health Organization* (WHO) data, the prediction of incremental rate of DM patient in Indonesia will rise up from 8.4 million in 2000 up to 21.2 million in 2030. Diabetic nephropathy is the complication that occurs in 20 – 40% of patients, thus can make the patient fall to chronic kidney disease (CKD).[1] *The Eureopean Diabetes* (EURODIAB) *Prospective Complications Study Group* showed the data that 12.6% Diabetes Mellitus Type 1 develops nephropathy, meanwhile the Diabetes Mellitus Type II group shows the rate is about 33% in 5 years after 10 years diagnosis. The development of nephropathy among diabetes patient consists of 3 phases, that is: normoalbuminuria, microalbuminuria, and macroalbuminuria. The progressivity is higher in Diabetes Mellitus Type 2 cases, since the Advanced Glycated End products production is higher than Diabetes Mellitus Type 1 group. It was also stated that genetic factors, over intake of the calorie, and inactive physical activity could contribute to the progression of glomerular damage that occurs in diabetic nephropathy.[1,2]

Diabetic Nephropathy could be diagnosed by performing albuminuria test using 24 hours urinary protein excretion. It could be established if urinary albumin excretion is over 30 mg in a day from 2 of 3 episodes in 3 – 6 periods of time, without no other albuminuria cause found. But it is not easy, since the compliance of patient is often bad. other collection method is needed to make it safer and easier, one of the method is calculation of albumin in random urine. Microalbuminuria or presence of albumin

* Corresponding author: rajanafiah@gmail.com
[1] Department of Clinical Pathology Faculty of Medicine, Universitas Padjadjaran, Bandung, Jawa Barat, Indonesia

in urine could predict the progressivity of diabetic nephropathy. Long standing hyperglycemia such as in diabetes mellitus could cause non-enzymatic glycation in many molecules such as hemoglobin, albumin, Low Density Lipoprotein (LDL), High Density Lipoprotein (HDL), IgG, IgM, collagen, insulin and histone Glycation process of the protein is linkage between glucose and amino group protein that occurs without enzymatic process. This is why the changes of those protein structures could happen. This process takes role in micro and macrovascular complication changes by forming Advanced Glycation End Products (AGEs)

Glycemic control assessment is very important to prevent the complication of diabetes mellitus. Glycemic control is divided into long term glycemic control (done in 2-3 months), such as HbA_{1c}; intermediate term control (2-3 weeks) such as fructosamine and glycated albumin; and short term control (3-5 days) such as 1,5-*anhydroglucitol* (1,5-AG). HbA1c measurement is already done to estimate long term glucose control clinically. Role of HbA1c was also used as a criteria of DM diagnosis according to *American Diabetes Association* (ADA) and *World Health Organization* (WHO) and PERKENI. Glycated albumin is a ketoamine that been formed by linkage between albumin and glucose by non-enzymatic oxidation reaction. Glycated albumin level in normal population is 11-16%, with half life is about 2-3 weeks, shorter than erythrocyte, and GA level changes is faster than erythrocyte life, so the level fluctuates faster than HbA1c. It is beneficial in monitoring therapy on DM patient, also in patient with anemia or renal failure, where the hemoglobin level could drop due to erythropoietin deficiency. Serum glycated albumin measurement is not influenced by renal anemia, so thus the parameter could be used as glycemic control examination in diabetic nephropathy patient, such as: first stage (glomerular hyperfiltration), second stage (microalbuminuria), third and fourth stage (macroalbuminuria, with or without hypoalbuminemia), and end stage (renal failure). Diabetic nephropathy patient in more than third stage could have been hypoalbuminemia due to proteinuria, and increasing albumin excretion and metabolism so thus glycated albumin level could be lower than normal. Meanwhile in fourth stage diabetic nephropathy is often followed by renal anemia that causes HbA1c becomes lower, due to shortening life span of erythrocyte, that is why this test is more useful to estimate glucose control in late stage nephropathy. The aim of this study is to compare the serum glycated albumin level in Diabetes Mellitus patient among those with normoalbuminuria, microalbuminuria, and macroalbuminuria.[4,5]

MATERIAL AND METHODS

Subjects of the Study

This is a comparative cross-sectional research, with subject collected in this research is Diabetes Mellitus patients that visit outpatient clinic in Endocrinology Division, Internal Medicine Department, RSUP Hasan Sadikin. The patient is asked the willingness to participate in this study, and then sign informed consent after explanation. According to estimation of sample size, it was found that each group should have 32 subjects, so minimum subjects that participate in this study is 96 subjects, with additional 10% data loss, so researchers decided to look for minimum 106 subjects in this research.

Sample Collection and Processing

Subject who has signed informed consent then was interviewed to obtain clinical data. Blood was obtained by phlebotomy in fossa cubiti by vacutainer inside plain tube for 2 mL and was let to coagulate in 30 minutes, then centrifuged for 10 minutes. Serum that has been processed then separated and collected in microtube plastic, and stored in -20^{o}C until the amount of research subject is fully obtained. Procession of this sample was conducted in Laboratory Installation of Hasan Sadikin Hospital, Bandung. In this research, the researcher performed glycated albumin, microalbuminuria, and urinary creatinine measurement. The glycated albumin level in serum is measured with colorimetric enzymatic method with sample from blood serum. The glycated albumin serum is stabile in 2-8°C for 7 days, in -20°C for 1 months, and if stored in -80°C up to 6 months. The urinary albumin and creatinine could be performed with spectrophotometer by using turbidimetry for urinary albumin, and using enzymatic colorimetric for urinary creatinine.

Statistical Analysis

All data collected (primary or secondary data) in this research then analyzed with Statistical Product and Service Solution (SPSS) for windows version 20.0. The data collected from this research underwent normality testing to know the dispersion of the data, then continued with multivariate analysis, to know the comparation among each groups of the research subject

RESULTS

In this research period, 105 subjects that fully meets criteria of this research are collected. Those subjects is T2DM that underwent medication from Endocrinology outpatient clinic, Department of Internal Medicine, Hasan Sadikin Hospital. Primary data obtained from the patient is urinary albumin per creatinine ratio (uACR) and serum glycated albumin (GA). Other parameters such as age, body mass index, duration of medication, type of medication, compliance to therapy was obtained from direct interview of subjects and medical records data.

All data collected, including uACR and GA was performed Kolmogorov Smirnov test, to see the normality of data, and the result showed that the data did not normally distributed, so those data will be presented by median and range.

Characteristic of the research subject including: age of the subject, duration of Diabetes Mellitus, body mass index, type of therapy, comorbidity, compliance of therapy, uACR and GA value is presented in the next table (Table 1)

Tabel 1 Characteristic of Research Subject (n = 105)

Variables	n(%)	Mean (SD)	Median (min-maks)
Age (Years)		60 ± 12	
25 - 34	2 (1,9)		
35 - 44	7 (6,7)		
45 - 54	22 (20,9)		
55 - 64	34 (32,4)		
65 - 74	30 (28,6)		
75+	10 (9,5)		
Sex			
Male	54 (51,4)		
Female	51 (48,6)		
Body Mass Index		25,79 ± 3,9	
normal	46 (43,8)		
overweight and *obese*	59 (56,2)		
Duration of Disease			6 (1 - 15)
<5 years	42 (40)		
5-10 years	49 (46,7)		
>10 years	14 (13,3)		
Comorbidity			
Hypertension	85 (80,9)		
Dyslipidemia	73 (69,5)		
Overweight and Obesity	59 (56,2)		
Compliance of Therapy			
Good	71 (67,6)		
Not Good	34 (32,4)		
Type of therapy			
Oral Hypoglycemic agent	69 (65,7)		
Insulin	21 (20,0)		
Both	15 (14,3)		
u-ACR			101 (1,0 - 3645,0)
Glycated Albumin level (%)			19,19 (12,1 - 47,2)
Normal (11%-15%)	26 (24,7)		
Increased (>15%)	79 (75,3)		

Note: Numerical data is presented with mean, standard deviation, range(min-max), and median. Categoric data is presented by proportion. The data that distributed normally is presented in mean (SD), but data with abnormal distribution is presented with median (min-max) u-ACR : *urinary* Albumin Creatinine Ratio; SD = standard of deviation

From the following Table 2, the researcher performed comparative analysis in some parameters according to degree of albuminuria in research subject,

Tabel 2 Characteristic of Research Subject Based on Albuminuria Degree

Variables	Albuminuria Degree			p-value
	Normoalbuminuria (n=35)	Microalbuminuria (n=35)	Macroalbuminuria (n=35)	
Age (years)	60.54 ± 8.67	59.23 ± 12.09	59.11 ± 12.46	0,874
Duration of T2DM (years)	4(1-12)	5(1-13)	8(1-15)	0,000**
Body mass index (kg/m^2)	24.509±3.986	26.25±3.19	26.64 ± 4.29	0,032**
Systolic blood pressure (mmHg)	130(110-160)	130(110-150)	140(110-160)	0,090
Diastolic blood pressure (mmHg)	80(80-90)	80(80-90)	90(80-100)	0,075
Glycated albumin(%)	15,93(12,07-21,89)	20,96(12,85-47,17)	23,14(13,58-46,05)	0,006 **
Serum Albumin (g/dL)	3,97 ± 0,39	3,71 ± 0,59	3,61 ± 0,44	0,000**

From the Table 2, from the analysis with Kruskal Wallis test it can be observed that there is some significant differences between two groups according to albuminuria degree, and found that there was statistically significant difference of serum glycated albumin between microalbuminuria and macroalbuminuria, but no statistically significant differences between microalbuminuria and macroalbuminuria group

DISCUSSION

Post Hoc Analysis from data above showed that there was significant differences in duration of diabetes mellitus between normoalbuminuria and macroalbuminuria group, and between microalbuminurian and macroalbuminuria, no significant differences was found. Body mass index (BMI) is significantly different only between normoalbuminuria and macroalbuminuria group, and albumin serum level is only significantly different between normoalbuminuria and macroalbuminuria group. Median of body mass index in Table 2 is higher at macroalbuminuria group compared to normoalbuminuria and macroalbuminuria, thus the higher BMI level, leads to worsening of kidney function. Meanwhile, albumin serum will decrease alongside with increasing level of albuminuria, this is caused by incremental rate of albumin excretion in urine is getting higher, due to destruction of renal structure. Reynolds et al showed that in normal blood glucose level, serum glycated albumin is negatively correlated with BMI, even the correlation is weak (r=0,25; p=0,027). Negative correlation is caused by high serum albumin turnover in obese patient, thus causing glycated albumin is relatively lower than plasma glucose. Besides, chronic systemic inflammation in obesity also takes role. Inflammation could increase rate of synthesis and increase albumin catabolism, so serum albumin exchange is also increased. From Table 2, higher BMI is found in macroalbuminuria compared to other groups. The higher BMI, the worse kidney function. Glycated albumin can influence biologic function of albumin which is interaction function or linkage between drugs and albumin. The affinity of OAH toward glycated albumin is lower than albumin. Some type of drugs that being affected by this are *glibenclamide, acetohexamide, tolbutamide, glicazide dan metformin*. [6,7,8]

According to uACR result, subject of this research is 105 subjects that is grouped into 3 group that is normoalbuminuria, microalbuminuria, and macroalbuminuria, with thes same amount in all

groups. The differences of uACR in normoalbuminuria group is 3 mg/g creatinine, microalbuminuria is 101 mg/g creatinine, and macroalbuminuria 808 mg/g creatinine. This results showed that the uACR result is getting higher in macroalbuminuria group, since the macroalbuminuria group has more extensive renal damage, the loss of albumin and creatinine excretion in urine is higher than any other group.[9,10]

There were significant differences of glycated albumin between normoalbuminuria and microalbuminuria group showed that glycated albumin is increasing in microalbuminuria group compared to normoalbuminuria. Prolonged hyperglicaemia can induced superoxide dismutase production from mitochondria that promotes disruption of metabolic pathway. The dysregulation induced formation of effector which directly disrupts endothelial glomerular cells damage, especially glycocalyx, then destroy intercellular communication, mainly between endothelial cells and podocytes. The damage occurs at microalbuminuria. In macroalbuminuria stage, the damage will be more extensive, signed by mesangial cellular expansion, structural changes, and loss of podocytes. Those phenomenon will increase glomerular permeability, thickening of glomerular basement membrane, then glomerulosclerosis.[11,12]

In this research, GA level in macroalbuminuria group is higher than microalbuminuria, also has better significancy, but not statistically significant. Other research at 2013 had found that GA level in T2D in macroalbuminuria group was lower than in microalbuminuria group, but not statistically significant. The differences among those research is possibly because the differences of research's population. [13]

From Table 2, it was found that glycated albumin level between microalbuminuria and macroalbuminuria is not statistically significant (p-value=0.137). it was caused by hypoalbuminemia (serum albumin level <3.5 g/dL) due to loss of urinary protein in T2DM with macroalbuminuria group. From the characteristic of research subject, it was found that proportion of T2DM with microalbuminuria with hypoalbuminemia is 25.7% (9/35), meanwhile T2DM with macroalbuminuria is 34.3% (12/35), with p-value = 0.660.[13,14]

Glycated albumin in serum is bound in total albumin protein so glycated albumin level is influenced by serum albumin level. Albumin homeostasis is influenced by synthetic and clearance of the albumin. Albumin clearance in plasma is the sum of albumin clearance from gastrointestinal, urinary tract, and catabolism component. Albumin clearance in urinary tract is about 6%, from gastrointestinal is about 10%, and other catabolism component is about the rest 84%. Meanwhile, albumin synthesis is influenced by osmotic colloid pressure and albumin level in plasma, but oncotic colloids pressure has stronger role in controlling the synthesis and compared to plasma albumin level. In macroalbuminuria, structural and functional changes in urinary tract occurred so the protein got loss from urine. [15]

Other result of this research also showed that there were positive correlation between uACR with serum glycated albumin, and statistically significant (r=0,621; p-value <0,001). The positive correlation between uACR with glycated albumin showed that the higher of uACR, the serum glycated albumin is also getting here, and otherwise vice versa.[10,16]

Hyperglycemia in DM could accelerate diabetic nephropathy process at the very early stage of disease, but the effect lessening from time to time alongside with deterioration of renal function if compared to other diabetic nephropathy progressivity, that is albuminuria and hypertension which has bigger role compared to other disorder of kidney function.[11,16]

CONCLUSION

There were statistically significant differences between T2DM patients with normoalbuminuria and microalbuminuria, and between normoalbuminuria and macroalbuminuria. But, no statistically significant differences between microalbuminuria and macroalbuminuria groups. This could be due to influence from urinary excretion of albumin in macroalbuminuria group

ACKNOWLEDGMENT

The researcher would like to biggest honour to Head of Department of Clinical Pathology

Faculty of Medicine Universitas Padjadjaran/RSUP Hasan Sadikin and Head of Study Program in Clinical Pathology Faculty of Medicine Universitas Padjadjaran/RSUP Hasan Sadikin, also with Outpatient laboratory MLT and Research laboratory MLT in Hasan Sadikin Hospital.

REFERENCES

1. American Diabetes Association. Standards of medical care in diabetes. Diagnosis and classification of diabetes mellitus [serial on the Internet]. 2016; 39(S1).
2. Ritz E, Schmieder RE, Pollock CA. Renal protection in diabetes: lessons from ONTARGET. Cardiovasc Diabetol. 2014 Oct 1;9:60. doi: 10.1186/1475-2840-9-60.
3. Fineberg D, Jandeleit-Dahm KA, Cooper ME. Diabetic nephropathy: diagnosis and treatment. Nature reviews Endocrinology. 2013 Dec; 9(12):713-23.
4. Onoa T, Kenichi Shikataa, Obikac M, Nobuyuki Miyataked, Koderaa R, Hirotaa D. Factors associated with remission and/or regression of mic- roalbuminuria in type 2 diabetes mellitus. Acta Med Okayama.2014;68(4):235-41.
5. Anguizola J, Matsuda R, Barnaby OS, Hoy KS, Wa C, DeBolt E, et al. Review: Glycation of human serum albumin. Clinica chimica acta; international journal of clinical chemistry. 2013 Oct 21;425:64-76.
6. Pleus, S., Freckmann, G., Schauer, S. Self-monitoring of blood glucose as an integral part in the management of people with type 2 diabetes mellitus. Diabetes Ther 13, 829–846 (2022). https://doi.org/10.1007/s13300-022-01254-8
7. Han JS, Lee MJ, Park KS, Han SH, Yoo TH, Oh KH, et al. Albuminuria as a risk factor for anemia in chronic kidney disease: Result from the KoreaN Cohort Study for outcomes in patients with chronic kidney disease (KNOW-CKD). PloS one. 2015;10(10):e0139747.
8. Neelofar K, Ahmad J. Review article : Amadori Albumin in Diabetic Ne- phropathy. Indian journal of endocrinology and metabolism. 2015;19(1):39-45.
9. Nathan DM, McGee P, Steffes MW, Lachin JM, Group DER. Relationship of glycated albumin to blood glucose and HbA1c values and to retinopathy, nephropathy, and cardiovascular outcomes in the DCCT/EDIC study. Diabetes. 2014 Jan;63(1):282-90.
10. Lee JW, Kim HJ, Kwon YS, Jun YH, Kim SK, Choi JW, Lee JE. Serum glycated albumin as a new glycemic marker in pediatric diabetes. Ann Pediatr Endocrinol Metab. 2013 Dec;18(4):208-13
11. Loeffler I, Wolf G. Epithelial-to-mesenchymal transition in diabetic nephropathy: fact or fiction? Cells. 2015 Oct 9;4(4):631-52. doi: 10.3390/cells4040631.
12. Smolock AR, Mishra G, Eguchi K, Eguchi S, Scalia R. Protein kinase C upregulates intercellular adhesion molecule-1 and leukocyte-endothelium interactions in hyperglycemia via activation of endothelial expressed. Arterioscler Thromb Vasc Biol. 2015 Feb;31(2):289-96. doi: 10.1161/ATVBAHA.110.217901. Epub 2015 Nov 15
13. Koga M, Murai J, Saito H, Otsuki M, Kasayama S. Evaluation of the glycated albumin/hba1c ratio by stage of diabetic nephropathy. Diabetol Int Journal. 2011;2:141-5.
14. Levitt DG, Levitt MD. Human serum albumin homeostasis: a new look at the roles of synthesis, catabolism, renal and gastrointestinalexcretion, and the clinical value of serum albumin measurements. International Journal of General Medicine. 2016;9:229-55.
15. Balamir I, Ates I, Topcuoglu C, Turhan T. Association of endocan, ischemia-modified albumin, and hscrp levels with endothelial dysfunction in type 2 diabetes mellitus. Angiology. 2018;69(7):609-616.
16. Ahmad T, Ulhaq I, Mawani M, Islam N. Microalbuminuria in type-2 diabetes mellitus; the tip of iceberg of diabetic complications. Pak J Med Sci. 2017 May-Jun;33(3):519-523. doi: 10.12669