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RESEARCH

# GLYCATED ALBUMIN AND HBA1C IN DIABETIC NEPHROPATHY

*(Albumin Glikat HbA1c dan Penyakit Nefropati Diabetik)*

**Elvan Dwi Widyadi, Jusak Nugraha, Ferdy Royland Marpaung**

***ABSTRAK***

*Nefropati diabetik merupakan komplikasi kronis yang sering terjadi di pasien penyakit Diabetus Melitus (DM), perubahan status glikemik harus diketahui lebih awal untuk mencegah komplikasi lebih lanjut. HbA1c sering digunakan sebagai pengendali glikemik jangka panjang (tiga/3 bulan). Albumin terglikasi merupakan tolok ukur baru yang dikembangkan untuk mengendalikan glikemik dalam waktu yang lebih singkat (2 minggu). Penelitian ini bertujuan untuk mengetahui kenasaban Albumin Glikat (GA) terhadap HbA1c di pasien nefropati diabetik. Dari 89 pasien DM yang diteliti, 34 didiagnosis sebagai diabetes nefropati. Kadar HbA1c diukur dengan metode Turbidimetric Inhibition Immunoassay (TINIA) dan GA diperiksa secara enzimatik. Analisis statistik dilakukan dengan menggunakan kenasaban Pearson, yang bermakna pada p<0,005. Cut off untuk GA: adalah 16% dan untuk HbA1c: 6,5%. Rerata GA: adalah 20,73% dan 7,42% HbA1c. Pada penelitian ini, dengan uji Pearson diketahui kenasaban yang baik antara GA dan HbA1c (r=0,785, p-nilai<0,0001). Albumin Glikat memiliki kenasaban yang kuat terhadap HbA1c. Oleh karena itu, GA dapat digunakan untuk mendeteksi indeks glikemik dalam jangka waktu singkat (2 minggu) di pasien pengidap nefropati diabetik.*

***Kata kunci:*** *Albumin glikat, HbA1c, nefropati diabetik, diabetes melitus*

**ABSTRACT**

Diabetic nephropathy is a chronic complication often occurring in Diabetes Mellitus (DM) patients. Glycemic control should be known early in order to prevent further complications. HbA1c is often used as a long term glycemic control (3 months). Glycated Albumin (GA) is a newly developed parameter for a short time glycemic control (2 weeks). The aim of this study was to know the correlation of GA and HbA1c in Diabetic Nephropathy patients. Out of 89 DM patients studied, 34 were diagnosed as Diabetic Nephropathy. HbA1c levels were measured by Turbidimetric Inhibition Immunoassay (TINIA), GA was determined by an enzymatic method. Statistical analysis was performed using Pearson correlation,which is significant for p<0.005. The cut off for GA: was 16% and for HbA1c: 6.5%. The mean of GA: was 20.73% and HbA1c 7.42%. In this study, by using Pearson's test, a good correlation between GA and HbA1c (r=0.785 p-value<0.0001) could be found. Glycated albumin is well correlated with HbA1c. Therefore, GA can be used to detect a short time glycemic index (2 weeks) in Diabetic Nephropathy patients.

**Key words:** *Glycated albumin, HbA1c, diabetic nephropathy, diabetes mellitus*

## INTRODUCTION

Diabetic Nephropathy is a chronic complication often occurring in DM patients. The accumulation of blood glucose for a long time will cause a non-enzymatic bond with protein through covalent bond which then re-unites with a similar compound causing a specific *crosslinked Advance Glycocylation End Products* (AGEs). The change in glycemic status must be known earlier to avoid further complications because the accumulation of AGEs will stimulate the increase of oxidative-stress in tissues, thus damaging the surrounding tissues.[1-3]

The diagnosing and supervising of diabetes at this stage and keeping the blood glucose level in normal limits must be taken into accordance with diabetes treatment. The epidemologically research results: *Collaboration Analysis of Diagnostic Criteria in* Europe (DECODE). *Funagata study, study prevent-*NIDDM reported that fluctuation of high blood glucose

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concentration will be endangering macrovascular disease, including heart and vascular disease in the first stage of glucose intolerance. Filtering and interfering diabetes in the early stage will be a great aid in preventing diabetes complications, heart and vascular disease. Other studies from *the Diabetes Control and Complication Study, United Kingdom Prospective Diabetes Study* and *Kumamoto study* show evidences that diabetic patients with bad glycemic level control will develop in diabetic nephropathy, neuropathy and retinopathy.[4]

HbA1c is often used as a long-term glycemic control by predicting glycemic status of patients in 3 months according to the erythrocyte life span. This observation has several drawbacks as it can cause false reading results either low or high results related to patients who suffer from disorders: kidney malfunction, anemia, some Hemoglobinopathy and pregnant mothers. Glycation or *Glycated Albumin* (GA) is a newly developed parameter to score the glycemic status. *Glycated Albumin* is expected to control the glycemic status especially with diabetic nephropathy complication in which GA in evaluating the glycemic level is not influenced by other hemoglobin and erythrocyte disorders. Besides this GA is able to provide a glycemic state earlier than 2 weeks, so the change of blood glucose fluctuation is easily evaluated.[1,2] This research was done to analyze the correlation of GA examination result towards HbA1c as the glycemic benchmark in a diabetic nephropathy patient.

## METHODS

Samples taken at random about 89 patients diagnosed as DM patients and examined either as Outpatients or Inpatients treated in the Dr. Soetomo Hospital Surabaya. Thirty four of them were diagnosed as diabetic nephropathy patients. Venous blood samples and serum were taken at the Clinical Pathology Laboratory Diagnostic Centre Building Dr. Soetomo Hospital Surabaya, the research was done from December 2014 to April 2015.

The samples were examined for HbA1c and GA. The method used to examine GA was by enzymatic method using reagent GA–L and performed using TMS 1024I*i* device.[6] The examination of HbA1c was done by *Turbidimetric Inhibition Immunoassay* (TINIA) method calculating HbA1c using a percentage with the equation: %HbA1c = 100 × HbA1c/Hb reagent used Hb1c flex® *cartridge* and reaction was done in Dimension instrument Rlx,[7] Cut *off* for GA: was 16%[8] and HbA1c: 6.5%,[9] other examinations of clinical chemistry (BUN, SC, albumin, hemoglobin, GDP, 2JPP) were done with an automated instrument.

The type of research was an analytical cross-sectional observation by using Pearson correlation statistical test. Statistical analysis was done by using *Excel* 20017 and *SPSS* 17. The correlation of GA and HbA1c value was tested by statistical parameter, Pearson's test. Among all of the tests, $p$ value<0.05 was considered as a significant difference. The range of GA and HbA1c was determined by calculating the mean ± 2 SB. *Cut off* for GA: was 16% and for HbA1c: 6.5%.

## RESULTS AND DISCUSSION

The demography data and clinical parameters of research samples are illustrated in Table 1.

Demography data were represented by age and gender while parameters measured were HbA1c, Glycated Albumin (GA), Fasting Blood Glucose (FBG), 2 Hours Post Prandial (2HPP), Serum *Creatinine* (SC), *Blood Urea Nitrogen* (BUN), proteinuria, albumin, hemoglobin. Demography data from research samples were of an average age of 57 years corresponding to the prevalence of diabetic patients with chronic complication.

**Table 1.** The characteristic of research subjects

| Parameter | Mean±SB |
|---|---|
| Male | 14 |
| Female | 20 |
| Age | 57.7±12 |
| HbA1c | 7.4±2 |
| GA | 20.7±8 |
| FBG | 122.9±97 |
| 2HPP | 242.8±124 |
| SC | 4.2±3 |
| BUN | 46.4±30 |
| Protein | +2 |
| Albumin | 2.38±1.1 |
| Hb | 7.3±5 |

Note: GA: *Glycated Albumin*, FBG: *Fasting Blood Glucose*, 2HPP: *2 Hours Post Prandial*, SC: *Serum Creatinin,* BUN: Blood Urea Nitrogen, Hb: Hemoglobin

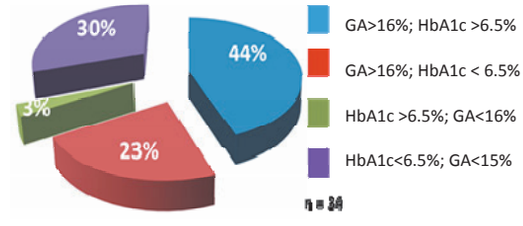


**Figure 1.** Distribution of HbA1c and GA examination results in Diabetic Nephropathy patients with a *cut off* HbA1c: 6.5% and *cut off* GA: 16%

**Table 2.** Distribution of GA and HbA1c check results towards the demographic distribution data

| | Sex | | Albumin | | Hemoglobin | | Proteinuria | |
|---|---|---|---|---|---|---|---|---|
| | **Male** | **Female** | **> 3** | **< 3** | **> 10** | **< 10** | **> +2** | **= +2** |
| | **n = 14** | **n = 20** | **n = 17** | **n = 17** | **n = 24** | **n = 10** | **n = 19** | **n = 15** |
| GA | 18.4±6.2 | 22.3±9.7 | 22.5±10.1 | 18.9±6.5 | 19.5±7.6 | 23.4±10.5 | 19.1 ±8.3 | 22.7± 8.8 |
| HbA1c | 6.7±1.8 | 7.8±2.5 | 7.9±2.5 | 6.9±1.9 | 7.5±2.3 | 7.22±2.3 | 6.7±1.4 | 8.3 ±2.9 |

**Table 3.** Correlation between GA & HbA1c by Pearson's test

| | R | P value |
|---|---|---|
| GA towards HbA1c | 0.785 | <0.0001 |

Besides this, the examination results of GA and HbA1c above the *cut off* also showed a chronic process of diabetic disease. Meanwhile, the mean increase of creatinine and the presence of proteinuria and anemia (Hb<10 g/dL) in this research were aimed as the diagnosis of Diabetic Nephropathy.

The results of GA and HbA1c by using the *cut off* GA 16% and HbA1c 6.5% as in figure 1 showed that there were 15 (44%) samples showing results for both GA and HbA1c above the *cut off*, Ten (30%) samples showed results for both GA as well as HbA1c below the *cut off*. Meanwhile, 8 (23%) samples of GA were above the *cut off* and HbA1c below the *cut off* and 1 (3%) sample was below the *cut off* and HbA1c above the *cut off* GA.

The distribution of demographic data for diabetic patients, in Table 2 showed that the number of females were 20 subjects while the number of males was only 14 people, with at *cut off* GA: >16% and HbA1c: >6.5% the distribution of mean results of GA and HbA1c in diabetic patients with different concentrations of albumin, hemoglobin and proteinuria resulted in a value above the *cut off*.

In this research, the correlation between the results of GA and HbA1c was calculated by Pearson's Test to see whether a correlation existed between GA and HbA1c or not in predicting the levels of glycemic index in nephropathy diabetic patients. Table 3 shows the result of Pearson Test as r=0.785 and a p value=<0.0001 which was very significant.

The correlation between GA and HbA1c as shown in Figure 2 at the intersection of the sample and the slope with r=0.875 and the p value of <0.001 showed a significance.

The study data were taken randomly from 89 samples of patients diagnosed with diabetes, 34 of them were patients with diabetic nephropathy as shown in Table 1. The average age of the samples was 57±12 with 20 females more than 14 males, at this age

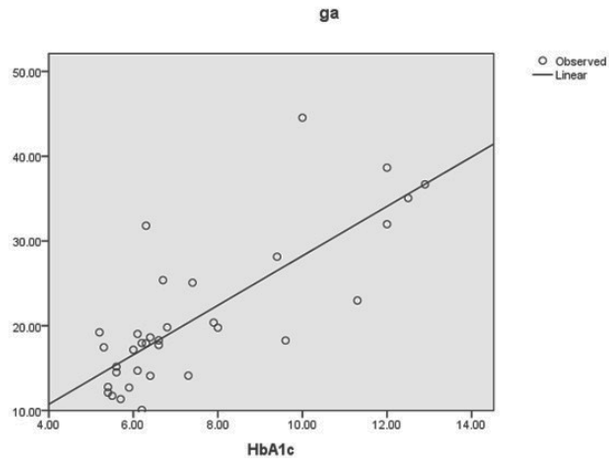


**Figure 2.** The correlation between GA and HbA1c

there is a tendency resulting in a degeneration process that will naturally form glycated-protein. However, this process will be more developed in patients with diabetes.[9] The average result of GDP examination and the increased 2JPP showed high levels of blood glucose in patients with diabetes corresponding to increasing levels of average GA and Hb1c with a cut off value as a measurement of glycemic as well as showing the process of chronic hyperglycemia in diabetic patients.[3] The increased creatinine followed by the increasing levels of blood glucose would activate oxidative stress in type 2 diabetic patients,[3,10] resulting in decreased renal function, as explained by Terawaki[11] and Huang *et al*.[12] who reported that the significant increase of creatinine was correlated to the increasing oxidative stress in patients with chronic renal diseases and patients in the final stage. Diabetic nephropathy disease was characterized by the average result of examining SC, BUN and proteinuria of 4.25±3.05; 46.41±30.36. The proteinuria in this case was shown by micro albuminuria, an early marker of renal involvement in type 2 diabetes with complications.[13]

The results of GA and HbA1c examination in Table 2 showed that the increase of both corresponded to the increasing age of 57.78±12 since the incidence of diabetes is most commonly found at that age. The

gender factor in this study indicated that the results of GA and HbA1c examination were higher in females than in males (see Table 2). In previous studies, HbA1c levels in males were higher than in females as reported by The Atherosclerosis Risk in Communities (ARIC) study.[14] This may be caused due to the fact that there were more samples of females than males. The result of GA examination could be influenced by the concentration of albumin, which in this study was shown in Table 2 from the GA examination of 22.5±10.1 in the albumin samples with more than 3 g/dL. Meanwhile, the GA examination of 22.7±8.8 with proteinuria 2+ was greater than the GA examination of 18.9±6.5 with an albumin of less than 3. The result of GA examination of 19.1±8.3 with proteinuria of more than 2+ corresponded to the limitations of GA examination towards the existing proteinuria which was massive such as in nephrotic syndrome and chronic renal disease stage 3 or 4 followed by decreasing levels of albumin.[14] The result of HbA1c examination could be influenced by Hemoglobin levels; meanwhile in this study, the average result of both HbA1c levels examination in Hb less than 10 mg/dL and more than 10 mg/dL showed no significant difference of 7.5 mg/dL and 7.22 mg/dL.

Glicated albumin and HbA1c as the results of formation changes of albumin serum or the age of red blood cells from non-enzymatic glycation are influenced by diseases or medications such as: liver, severe renal, thyroid, anemia, malnutrition, malignancy, pregnancy, and steroid treatment.[2] The result of GA and HbA1c examination in this study has each characteristic as shown in Figure 1. The result of GA and HbA1c examination was more than 16% *cut-off* and 6.5% as many as 15 samples of 34 (44%) which were examined. GA was more than 16%. HbA1c was less than 6.5% in 8 (eight) samples out of 34 (23%). Lately, many studies compared the accuracy of GA with HbA1c as an indicator for control of long-term glycation in patients with CKD either pre dialysis or dialysis. GA has a good and meaningful correlation towards the patient's blood glucose with/without CKD stage 4 and 5 and furthermore, GA was said to be a better indicator in monitoring glycation in patients with CKD with/without dialysis.[15] HbA1c has been proven to be reliable and can be used as a marker for predicting the course of the disease in diabetic patients. However, it is invalid if applied to patients with nephropathy diabetes. Some conditions in patients with CKD could influence the concentration of HbA1c, so it could result in both false low and high results. Besides blood glucose, HbA1c is influenced by several other factors, such as the age of erythrocytes, recombinant erythropoietin treatment (rHuEpo), uremia, blood transfusion, and hemodialysis. The faster the circulation of erythrocytes, the shorter the exposure of glucose will be, which in turn will decrease the levels of non-enzymatic glucose binding to the hemoglobin. This process will decrease the HbA1c, thus in patients with CKD the age of red blood cells will be shortened. The low level of HbA1c can be observed by monitoring the concentration of blood glucose.[16] GA was less than 16%, and HbA1c was more than 6.5% in one subject out of 34 (3%), this condition was caused due to the very low concentration of albumin 1.6g/dL and accompanied with proteinuria 3+. It is stated that the limitation of GA examination is related to a massive proteinuria such as in nephrotic syndrome and stage 3 or 4 of chronic kidney disease which are accompanied by a decrease of albumin level. Glycated albumin less than 16% and HbA1c less than 6.5% occurred in 10 out of 34 samples (30%) caused by the success of monitoring and treating diabetes, so that the glycemic status of diabetic nephropathy patients was still within the normal limits.

The analysis result of statistical test by Pearson in Table 3 showed a very significant correlation between GA and HbA1c in monitoring glycemic status in diabetic nephropathy patients with r=0.785 and –p value<0.001 (thus very significant). This also appears in Figure 2 in the equation of linier regression showing a very strong correlation by contacting with slope line.

The limitation of this research was that it was an observation by studying the correlation between GA and HbA1c, the number of samples in this research was not counted statistically, so they could not be used as a generalization of study results, the parameter used in this research was not adequate enough to support the existence of diabetic nephropathy disease such as that there was no creatinine clearance data to evaluate the function of kidney. Diagnosis of diabetic nephropathy generally does not use the staging of renal damage.

## CONCLUSIONS

GA has a strong correlation with HbA1c. Therefore, GA could be used to detect glycemic index in a short period (2 weeks) in diabetic nephropathy patients.

## REFERENCES

1. Lee EY, Lee BW, Kim D, Lee YH, Lee HC. Glycated albumin is a useful glycation index for monitoring fluctuating and poorly controlled type 2 diabetic patients, Acta Diabetol 2011; 48(2): 167–172.

2. Takahashi S, Uchino H, Shimizu T, Kanazawa A, Tamura Y, Sakai K, Watada H, Hirose T, Kawamori R, Tanaka Y. Comparison of Glycated Albumin (GA) and Glycated Hemoglobin (Hb A1c) in Type 2 Diabetic Patients: Usefulness of GA for evaluation of Short-Term Change in Glycemic Control. Endocrine Journal 2007; 54(1): 139–144.
3. Makita Z, Radoff S, Rayfield EJ, Yang Z, Skolnik E, Delaney V, Friedman EA. Advanced Glycosylation End Products in Patients with Diabetic Nephropathy, The New England Journal of Medicine Sept 19, 1991; 325(12): 836–842.
4. Furusyo N, Koga T, Ai M, Otokozawa S, Kohzuma T, Ikezaki H, Schaefer EJ, Hayashi J. Utility of glycated albumin for the diagnosis of diabetes mellitus in a Japanese population study: results from the Kyushu and Okinawa Population Study (KOPS), Diabetologia 2011; 54(12): 3028–36.
5. Nazar CMJ. Diabetic nephropathy; principles of diagnosis and treatment of diabetic kidney disease, J Nephropharmacol. 2014; 3(1): 15–20.
6. Asahi Kashei Pharma: Insert kit GA-L Reagent, 2013.
7. Siemens Dimension: insert kit flex ® reagent cartridge HbA1c, 2013.
8. The Japan Diabetes Society: Diabetes Treatment Guide 2002–2003, Bunkodo Co. Ltd, 2002; 7.
9. World Health Organization: Use of Glycated Hemoglobin (HbA1c) in the Diagnosis of Diabetes Mellitus, Geneva 27, Switzerland, WHO, 2011; 6.
10. Monnier L, Mas E, Ginet C, Michel F,Villon L, Cristol JP, Colette C. "Activation of oxidative stress by acute glucose fluctuations compared with sustained chronic hyperglycemia in patients with type 2 diabetes," Journal of the American Medical Association, 2006; 295(14): 1681–87.
11. Terawaki H, Yoshimura K, Hasegawa T, Negawa T, Yamada K, Matsushima M, Nakayama M, Hosoya T, Era S, "Oxidative stress is enhanced in correlation with renal dysfunction: examination with the redox state of albumin," Kidney international, 2004; 66(5): 1988–93.
12. Huang KC, Yang CC, Lee KT, Chien CT, "Reduced hemodialysis-induced oxidative stress in end-stage renal disease patients by electrolyzed reduced water," Kidney International, 2003; 64(2): 704–714.
13. Remuzzi G, Schieppati A, Ruggenenti P, Nephropathy in Patients with Type2 Diabetes, N Engl J Med, 2002; 346(15): 1145–51.
14. Furusyo N, Hayashi J, Glycated albumin and diabetes mellitus, Biochim. Biophys. Acta (2013), Dec; 1830(12): 5509–14.
15. Vos FE, Schollum JB, Walker RJ. In-depth Clinical Review Glycated albumin is the preferred marker for assessing glycemic control in advanced chronic kidney disease. Nephrology Dialysis Transplantation Plus 2011; 4(6): 368–375.
16. Ly J, Marticorena R, Donnelly S. Red blood cell survival in chronic renal failure. Am J Kidney Dis 2004; 44(4): 715–719.