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CORRELATION OF ADVANCED GLYCATION END PRODUCTS WITH URINARY ALBUMIN CREATININ RATIO IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

(Kenasaban Kadar Advanced Glycation End Products dengan Rasio Air Kemih Albumin Kreatinin di Pasien Diabetes Melitus Tipe 2)

Debie Anggraini[1], Rismawati Yaswir[2], Lillah[2], Husni[2]

ABSTRAK

Nefropati diabetik merupakan komplikasi mikrovaskular di pasien Diabetes Melitus (DM) yang menyebabkan End Stage Renal Disease (ESRD). Proses glikasi non-enzimatik asam amino bebas di hiperglikemia kronis menghasilkan advanced glycation end-products (AGEs). Advanced glycation end-products dimetabolisme di ginjal sehingga terjadi penumpukan yang memicu kerusakan glomerulus ginjal. Advanced glycation end-products terbukti berperan dalam perjalanan penyakit nefropati diabetik. Pemeriksaan mikroalbuminuria dengan rasio air kemih albumin kreatinin merupakan pemeriksaan yang disarankan untuk mendeteksi nefropati lebih awal. Tujuan penelitian ini untuk mengetahui kenasaban kadar AGEs dengan rasio air kemih albumin kreatinin di pasien DM tipe 2. Penelitian ini menggunakan metode analitik dengan desain potong lintang di 30 orang pasien DM tipe 2 yang memenuhi patokan kesertaan dan nonkesertaan serta melakukan pemeriksaan darah di Instalasi Laboratorium Sentral RSUP Dr. M. Djamil Padang masa waktu Mei 2015-Maret 2016. Pemeriksaan kadar AGEs dilakukan dengan metode sandwich ELISA. Pemeriksaan mikroalbumin air kemih dilakukan dengan metode imunoturbidimetri. Data dianalisis dengan uji kenasaban Spearman, bermakna jika p<0,05. Rerata kadar AGEs di DM tipe 2 adalah 1052,18±750,25 ng/L. Rerata nilai rasio air kemih albumin kreatinin di pasien DM tipe 2 adalah 23,77±16,58 mg/g. Uji kenasaban Spearman menunjukkan kenasaban sangat kuat antara kadar AGEs dan rasio air kemih albumin kreatinin dengan nilai r=0,85 dan nilai p<0,05. Terdapat kenasaban sangat kuat antara kadar AGEs dengan rasio air kemih albumin kreatinin di DM tipe 2.

Kata kunci: *Advanced glycation end-products, rasio air kemih albumin kreatinin, diabetes melitus tipe 2*

ABSTRACT

Diabetic nephropathy is a microvascular complications of Diabetes Mellitus (DM), that progresses to End Stage Renal Disease (ESRD). Non-enzymatic glycation of free amino acids in chronic hyperglycemia produces advanced glycation end-products (AGEs). Advanced glycation end-products are metabolized in kidney and the accumulation induced glomerulus injury. Advanced glycation end-products is proven in pathogenesis of diabetic nephropathy. Microalbuminuria assay using Urinary Albumin Creatinine Ratio (UACR) is recommended to detect early diabetic nephropathy. The aim of this study was to determine the relationship of AGEs levels with UACR in patients with type 2 diabetes mellitus. This was an analytical study with cross-sectional design in 30 patients type 2 diabetes who met the inclusion and exclusion criteria and conducted blood tests at the Central Laboratory Instalation of Dr. M. Djamil Hospital Padang in May 2015–March 2016. Level of AGEs was performed by sandwich-ELISA method. Microalbuminuria was performed by imunoturbidimetri method. Data was analyzed by Spearman correlation test, significant if p<0.05. The mean levels of AGEs in patients with type 2 diabetes mellitus was 1052.18±750.25 ng/L. The mean value of UACR in patients with type 2 diabetes mellitus was 23.77±16.58 mg/g. Spearman correlation test showed a very strong correlation between the levels of AGEs and UACR with r=0.85 and p<0,05. There was a very strong correlation between AGEs levels with UACR in type 2 diabetes mellitus.

Key words: Advanced glycation end-products, urinary albumin creatinine ratio, diabetes mellitus type 2

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INTRODUCTION

Diabetes mellitus (DM) is a disease with abnormal clinical symptoms caused by hyperglycemia with the characteristics polyuria, polydypsia and polyphagia.[1] All of the symptoms in DM can be occurred because secretion disorders, impaired insulin function or both.[2] The incidence of DM in the United States increased by 49% (from 4.9 to 7.3% of the total population). Approximately 284 million people in worldwide have been diagnosed with diabetes in 2011, this amount is expected to increase two-fold by the year 2030.[2,3]

Diabetic nephropathy is a microvascular complication occurring in 20–40% of diabetic patients that causes End Stage Renal Disease (ESRD) in one-third of cases.[4] Chronic hyperglycemia in patients with DM can cause the non-enzymatic glycation of free amino acids which change the structures and functions, it will lead to renal glomerular damage because of enhancement of Advanced Glycation End Products (AGEs).[5]

Advanced glycation end-products are products of nonenzymatic protein glycation with diverse chemical structures. Proteins are modified, the aldehyde group of glucose reacts with amino group in protein, forming a glycosylated product which will undergo a series of reactions with NH2, form crosslinks proteins and produce AGEs.[6] Advanced glycation end-products are proven to play a role in the pathogenesis of diabetic nephropathy in Patients with ESRD. Kidney is a place of AGEs metabolism and accumulated damages because of AGEs that cause diabetic nephropathy.[7]

There was a correlation between serum levels of AGEs and urinary albumin creatinine ratio (UACR) in patients with type 2 diabetes mellitus (r=0.398).[8] Morcos et al.[9], concluded that AGEs level was positively correlated with microalbuminuria (UACR) in patients with type 2 diabetes mellitus (r=0.7).[9]

Clinically, the most important screening tool to detect early diabetic nephropathy is detection of microalbuminuria. Measurement of urinary albumin excretion rate 24-hour urine samples has been used as the gold standard for the evaluation of albuminuria, but this test has high value bias because the time of sample collection is inaccurate. Therefore, to obtain accurate and consistent results, the American Diabetes Association (ADA) and the National Kidney Foundation (NKF) recommends the measurement of UACR in random urine samples to diagnose microalbuminuria.[10,11]

There are no studies about the relationship between levels of AGEs and UACR in Indonesia. This study will investigate whether there is a correlation between AGEs level with UACR in patients with type 2 diabetes mellitus at the Dr. M. Djamil Padang Hospital. This study aimed to prove the correlation between AGEs level with UACR in patients with type 2 diabetes mellitus.

METHODS

This study was approved by the Ethics Committee of the Faculty of Medicine Andalas University. This study was an analytical study with cross-sectional design and conducted at the Central Laboratory Installation of Dr. M. Djamil Padang Hospital and Biomedical laboratory of Medical Faculty, Andalas University from May 2015 to March 2016.

Population of this study were all patients who had been diagnosed with type 2 diabetes by an internist of Dr. M. Djamil Hospital Padang. The samples of this study were part of population who met the inclusion and exclusion criterias. Inclusion criterias: patients who had been diagnosed with type 2 diabetes by an internist, aged over 18 years, fasting 8–10 hours before the test and agreed to participate in the research. Exclusion criterias: patients who had been diagnosed with type 2 diabetes with impaired renal function (ureum >50 mg/dL, creatinine >1.1 mg/dL) and real proteinuria.

Level of AGEs was performed by sandwich-ELISA method, about 3 mL of venous blood was obtained from cubital vein phlebotomy inserted into vacuum tubes without anticoagulant and allowed to clot at room temperature for 30 minutes and then centrifuged at 3000 rpm for 15–20 minutes. Serum was inserted into aliquots and stored at a temperature of –20°C (stable 1 month) or –80°C (stable 6 months). Avoid repeated thawing. AGEs level in normal subjects was ≤ 1,245.8 ng/L and in diabetic patients 1,245.8 ng/L.[8]

Microalbuminuria was performed by imunoturbidimetri method, the samples were fresh urine specimens inserted into an urine tube and centrifuged at 1500 rpm for 10 minutes, the supernatant was taken and stored at a temperature of –20°C (stable for 6 months). Data was analyzed by Spearman correlation test, significant if p<0.05. Urine albumin creatinin ratio in normoalbuminuria <30 mg/g, microalbuminuria 30–300 mg/g and macroalbuminuria >300 mg/g.[2]

RESULTS AND DISCUSSION

A cross-sectional study was conducted on 30 type 2 DM patients, who were outpatients of the Outpatient

Clinic Department of Internal Medicine Dr. M. Djamil Hospital. Research samples were patients who met the inclusion and exclusion criterias and underwent blood tests at the Central Laboratory Installation Dr. M. Djamil Hospital Padang from May 2015 to March 2016. The examined parameters included the levels of urine albumin and creatinine urine, UACR and serum AGEs levels. The data were analyzed by using a computer program.

The research subjects consisted of 12 males (40%) and 18 females (60%). The mean age of subjects was 57.8±8.5 years, with a lifespan of 38–71 years. A total of 26.7% of patients were diagnosed with type 2 DM with microalbuminuria and 73.3% of patients were diagnosed with type 2 diabetes normoalbuminuria. The research subjects consisted of 30 samples with the following description of the basic characteristics (Table 1).

Table 1. Basic characteristics of subjects

Variable	n (%)	Mean±SD
Gender		
Males	12 (40)	
Females	18 (60)	
Age (years)		57.8±8.5
Urine Albumin Excretion		
Microalbuminuria (g/dL)	8 (26.7)	
Normoalbuminuria (g/dL)	22 (73.3)	

The mean of UACR in subjects was 23.77±16.58 mg/g with the lowest value of 7.21 mg/g and the highest value of 77.10 mg/g. The mean levels of AGEs were 1052.18±750.25 ng/L with the lowest level of 365.46 ng/L and the highest levels of 3252.45 ng/L (Table 2).

Table 2. Urinary albumin creatinin ratio and serum advanced glycation end-products

Variable	Mean±SD	Lowest level	Highest level
UACR (mg/g)	23.77±16.58	7.21	365.46
AGEs (ng/L)	1,052.18±750.25	77.10	3252.45

The mean of UACR in subjects diagnosed with type 2 diabetes with microalbuminuria was 45.68±16.63 mg/g while the mean of UACR in subjects diagnosed with type 2 diabetes with normoalbuminuria was 15.8±6.15 mg/g. The mean levels of AGEs in subjects with type 2 diabetes mellitus with microalbuminuria were 1959.20±989.17 ng/L, while AGEs level in subjects with type 2 diabetes mellitus with normol-buminuria was 722.36±154.24 ng/L (Table 3).

Table 3. Urinary albumin creatinin ratio and serum advanced glycation end-products based urinary albumin excretion

Variable	Microalbuminuria (Mean±SD)	Normoalbuminuria (Mean±SD)
UACR (mg/g)	45.68±16.63	15.80±6.15
AGEs (ng/L)	1,959.20±989.17	722.36±154.24

Correlation between serum AGEs and UACR in patients with type 2 diabetes statistically tested by Spearman correlation test showed a very strong correlation (r=0.85; p<0.001) (Table 4) (Figure 1).

Table 4. Corelation serum AGEs and UACR in type 2 diabetes

	AGEs
UACR	r=0.85
	p<0.001

The results of this study showed a very strong positive correlation between serum AGEs level with UACR (r=0.850) and were significant (p<0.001). Morcos et al.[9], obtained a strong correlation between AGEs level and UACR (r=0.7), significant (p<0.001) in patients with DM type 2.[9] Khauly et al.[8] obtained a correlation between AGEs level and UACR (r=0.398), significant (p<0.001) in type 2 diabetes.[8]

Differences between age, gender and the duration of type 2 DM in researched subjects caused differences in outcomes of this study with several other studies.[8] The long duration of type 2 DM and their treatment in glycemic control will affect whether or not the incidence of chronic hyperglycemia in type 2 DM was shown and thus will influence the serum AGEs level.

Chronic hyperglycemia causes non-enzymatic glycation with amino acids that will form irreversible AGEs.[12] Advanced glycation end products not only produced a state of hyperglycemia but also were influenced by oxidative reactions that occured in the cells.[13,14] Reactive Oxygen Species (ROS) plays a role in the formation of AGEs and mediates the effects AGEs on target tissues. The relationship between clinical complications of type 2 DM and oxidative stress occured in the elevated levels of AGEs.[15,16]

AGEs bound on the glomerular basement membrane in diabetic patients causes a decrease in binding with heparan sulfate proteoglycan (HSPG/stimulator of matrix molecules in blood vessel) causing loss of selectivity of the barrier electric charge so that large molecular protein will pass into the tubular lumen. AGEs binds with RAGE receptor on mesangial cells stimulating the secretion of platelet-derived growth
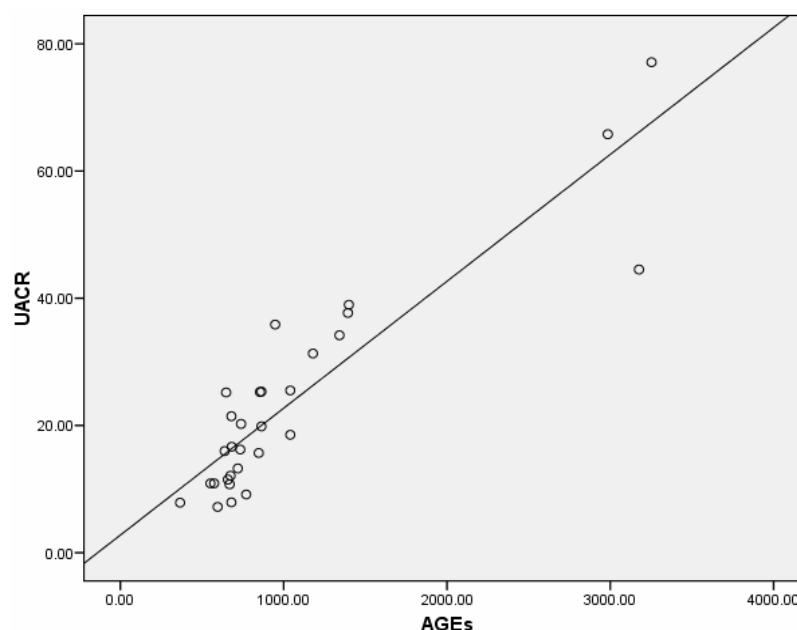
Figure 1. Linear curve of serum AGEs level and UACR in type 2 diabetes.

factor which will mediate the expansion of mesangium and glomerulosclerosis in diabetic nephropathy.[13,14,17]

This study and other studies supported the theory that AGEs elevated levels associate with progression of diabetic nephropathy. AGEs level becomes a reference in patients with type 2 DM to monitor and prevent the occurrence of diabetic nephropathy.

CONCLUSION AND SUGGESTION

There was a very strong correlation between AGEs levels with UACR in type 2 diabetes mellitus. A further study should be done by using normal subjects (control subjects) to determine the cut off levels of AGEs in normal individuals and patients with type 2 DM in Indonesia.

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