Vol. 23, No. 3, July 2017

p-ISSN 0854-4263
e-ISSN 4277-4685

INDONESIAN JOURNAL OF

Clinical Pathology and Medical Laboratory

Majalah Patologi Klinik Indonesia dan Laboratorium Medik

IJCP & ML (Maj. Pat. Klin. Indonesia & Lab. Med.)	Vol. 23	No. 3	Page 203-309	Surabaya July 2017	ISSN 0854-4263

Published by Indonesian Association of Clinical Pathologists

Accredited No. 36a/E/KPT/2016, Tanggal 23 Mei 2016

p-ISSN 0854-4263
Vol. 23, No. 3, July 2017 e-ISSN 4277-4685

INDONESIAN JOURNAL OF

CLINICAL PATHOLOGY AND MEDICAL LABORATORY

Majalah Patologi Klinik Indonesia dan Laboratorium Medik

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Accredited No. 36a/E/KPT/2016, Tanggal 23 Mei 2016

p-ISSN 0854-4263
Vol. 23, No. 3, July 2017 e-ISSN 4277-4685

INDONESIAN JOURNAL OF

CLINICAL PATHOLOGY AND MEDICAL LABORATORY

Majalah Patologi Klinik Indonesia dan Laboratorium Medik

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Printed by Airlangga University Press. (OC 252/08.17/AUP-A1E). E-mail: aup.unair@gmail.com
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Thanks to editors in duty of IJCP & ML Vol 23 No. 3 July 2017

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INDONESIAN JOURNAL OF
CLINICAL PATHOLOGY AND MEDICAL LABORATORY
Majalah Patologi Klinik Indonesia dan Laboratorium Medik

2017 July; 23(3): 275–280
p-ISSN 0854-4263 | e-ISSN 4277-4685
Available at www.indonesianjournalofclinicalpathology.or.id

RESEARCH

CORRELATION OF URINE N-ACETYL-BETA-D-GLUCOSAMINIDASE ACTIVITY WITH URINE ALBUMIN CREATININE RATIO IN TYPE 2 DIABETES MELLITUS

(Kenasaban Aktivitas N-Asetil-Beta-D-Glukosaminidase Air Kemih dengan Air Kemih Albumin Kreatinin Rasio di Diabetes Melitus Tipe 2)

Melly Ariyanti, Lillah, Ellyza Nasrul, Husni

ABSTRAK

Urine Albumin Creatinine Ratio (UACR) merupakan metode untuk mengukur mikroalbuminuria, petanda awal dan paling banyak digunakan untuk diagnosis nefropati diabetes. N-asetil-beta-d-glukosaminidase (NAG) air kemih adalah enzim lisosom dengan berat molekul besar sehingga tidak difiltrasi oleh glomerulus. Ekskresi NAG di air kemih sangat mudah terganggu terutama oleh filtrasi albumin. Tujuan penelitian ini untuk mengetahui kenasaban NAG air kemih dengan UACR di pasien DM tipe 2. Penelitian ini merupakan penelitian analitik dengan rancangan potong lintang terhadap 25 pasien DM tipe 2 yang berobat ke Poliklinik Endokrin Penyakit Dalam RSUP Dr. M. Djamil Padang. Penelitian dilakukan mulai bulan Januari 2016–September 2016. Albumin air kemih diukur dengan metode imunoturbidimetri, kreatinin air kemih dengan metode Jaffe dan NAG dengan Enzyme-Linked Immune Sorbent Assay (ELISA). Kenasaban Spearman dilakukan untuk mengetahui hubungan aktivitas NAG air kemih dan UACR. Penelitian ini terdiri dari laki-laki 68% dan perempuan 32%. Rerata umur 56,16±7,6 tahun dengan rentang 39–67 tahun. Kadar glukosa, ureum dan kreatinin serum berturut-turut, 148±49 mg/dL, 24±6,2 mg/dL dan 0,9±0,3 mg/dL. Normoalbuminuria sebanyak 80% dan mikroalbuminuria 20%. Median nilai UACR pasien DM tipe 2 adalah 6,02 (16,46) mg/g dengan rentang 1,53–119,41 mg/g dan rerata kadar NAG adalah 51,01±31,88 ng/mL dengan rentang 9,45–144,38 ng/mL. Uji kenasaban Spearman menunjukkan kenasaban yang kuat antara aktivitas NAG air kemih dengan UACR dengan r=0,614 dan p<0,05. Terdapat kenasaban yang kuat antara aktivitas NAG air kemih dengan UACR di pasien DM tipe 2.

***Kata kunci**: Diabetes melitus tipe 2, N-asetil-beta-d-glukosaminidase, urine albumin creatinine ratio*

ABSTRACT

Urine Albumin Creatinine Ratio (UACR) is method to measure microalbuminuria, it is the earliest and most commonly used marker of diabetic nephropathy (DN). N-acetyl-beta-d glucosaminidase (NAG) is a high molecular weight lysosomal enzyme that cannot pass into the glomerular ultrafiltrate. Excretion of NAG is easily pertubed specially by albumin filtration. The objective of this study was to analyze the correlation between NAG level with UACR in type 2 Diabetes Mellitus (DM). This was an analytical study with cross-sectional design in 25 type 2 diabetes mellitus outpatient in the Internal Medicine Department Dr. M. Djamil Hospital Padang. The study was conducted from January-September 2016. Urine albumin was measured by the immunoturbidimetry method, urine creatinine was measured by Jaffe method and urine NAG was measured by enzyme-linked immune sorbent assay (ELISA) method. Data were analyzed by Spearman correlation test, significant if p<0.05. This study consisted of 68% males and 32% females. Mean of age was 56.16±7.6 years old with a range 39–67 years old. Mean of serum glucose, Urea and creatinine level, were 148±49 mg/dL, 24±6.2 mg/dL and 0.9±0.3 mg/dL respectively. Normoalbuminuria was 80% and microalbuminuria 20%. Median value of UACR in type 2 DM was 6.02 (16.46) mg/g with a range of 1.53–119.41 mg/g and mean level of urine NAG was 51.01±31.88 ng/mL with a range of 9.45–144.38 ng/mL. Spearman correlation test showed a strong correlation between urine NAG with UACR in type 2 DM with r=0.614 and p<0.05. There was a strong correlation between urine NAG with UACR in type 2 DM.

Key words: N-acetyl-beta-d-glucosaminidase, type 2 diabetes mellitus, urine albumin creatinine ratio

Department of Clinical Pathology, Faculty of Medicine, Andalas University - Dr. M. Djamil Hospital, Padang, Indonesia.
E-mail: mellya77@yahoo.co.id

INTRODUCTION

Kidney disorder is a serious complication of Diabetes Mellitus (DM), estimated as in one-third of DM patients worldwide. Diabetes mellitus is associated with morbidity and mortality of cardiovascular disease as well as considered as the primary factor of End Stage Renal Disease (ESRD). Death in DM patients due to kidney disease (diabetic nephropathy) occurs more frequently as many as seventeen times than in non-DM patients. Therefore, it is necessary to detect kidney damage as early as possible so that the onset of nephropathy can be prevented or the progression of the disease may be delayed.[1-3]

A classical method used to diagnose diabetic nephropathy through proteinuria detection with urinalysis or decreased creatinine clearance is considered to be inappropriate since this method is not effective enough to prevent disease progression faster. Advanced laboratory examination technique has recently improved diabetes management. The presence of this laboratory examination technique can detect albumin at a relatively low level expressed in the form of urine albumin to creatinine ratio (UACR ie normoalbuminuria: <30 mg/g and microalbuminuria: 30-300 mg/g), making this parameter a preliminary marker and the most widely used in detecting the early stage of diabetic nephropathy.[1–3]

The progression of diabetic nephropathy in DM with microalbuminuria can be suppressed by good blood glucose level and blood pressure control through the administration of Angiotensin Converting Enzyme (ACE) inhibitors as antihypertensive drugs, but morphological changes actually have occurred earlier than the manifestations of microalbuminuria.[3] A research conducted by Robles-Osorioa *et al*.[4] found that 29.1–61.6% of patients with type 2 diabetes had already suffered kidney damage before microalbuminuria appeared. Thus, detecting the early-stage of diabetes nephropathy before the appearance of microalbuminuria becomes crucial to reduce progression to, improve outcomes and provide a better prognosis.[1,5]

Many new parameters actually have been widely studied, including urine proteins (except albumin) in detecting the early-stage of diabetic nephropathy. N-acetyl-beta-d-glucosaminidase (NAG) is the most widely discussed urine enzyme. This enzyme is a lysosomal enzyme found in all nucleated cells with the highest concentration present in the proximal tubule. The enzyme has a large molecular weight, so it is not filtered by the glomerulus. N-acetyl-beta-d-glucosaminidase (NAG) under normal circumstances is excreted in small amounts as a result of proximal tubular exocytosis process and is relatively stable against changes in pH, temperature, as well as endogenous inhibitors, such as urea and ascorbic acid in urine.[2,6,7] N-acetyl-beta-d-glucosaminidase examination is also quite easy to perform. There are several methods to assess its activities. Fluorometry was the first method to be used, introduced in 1960. Colorimetry and spectrophotometry have recently been the most widely used ones since they are very easy to use.[6]

N-acetyl-beta-d-glucosaminidase excretion, moreover, is very easily disturbed primarily by albumin filtration. Increased albumin filtration will increase proximal tubular reabsorption activity to prevent albumin excretion through urine. Next, the reabsorbed albumin will be degraded by lysosomes in proximal tubular cells into amino acids and then returned to the circulation, while lysosomes will dispose waste products and NAG into the urine through exocytosis. Increased NAG in urine, consequently, can indicate an increase in tubular activity indicating early renal impairment when tubular reabsorption capacity is still good.[1]

Furthermore, a research conducted by Kanauchi & Dohi[8] on biopsy in type 2 diabetes patients with microalbuminuria revealed that 57% of those patients had glomerulosclerosis and a very high NAG level ($p<0.001$), thus, NAG level can be used to predict the occurrence of glomerulosclerosis ($r = 0.734$, $p<0.001$). Similarly, a research conducted by Vlatkovic *et al*.[9] showed that NAG level in DM patients with normoalbuminuria elevated as many as three times in microalbuminuria and as many as twice in macroalbuminuria if compared to in healthy controls.

In addition, a research conducted by Bouvet *et al*.[10] on the clinical use of NAG in Argentina showed that there was a stronger correlation of NAG ($r=0.628$; $p <0.0001$) with UACR than with levels of blood glucose, HbA1c, serum creatinine and glomerular filtration rate. Meanwhile, a research conducted by Kim *et al*.[11] on the usefulness of NAG in patients with type 2 diabetes in South Korea argued that there was a moderate correlation of NAG with UACR ($r=0.458$, $p<0.001$). As a result, this research aimed to reveal the correlation of NAG with UACR in type 2 diabetes patients visiting the Dr. M. Djamil Hospital in Padang.

METHODS

This research was an analytical research with a cross-sectional design. This research was conducted

in the Central Laboratory Installation of Dr. M. Djamil Hospital in Padang, Prodia Laboratory in Padang and Biomedical Laboratory of Faculty of Medicine at Andalas University in Padang. This research was performed from January-September 2016. The research population were patients who had been diagnosed with type 2 diabetes by internists of the Dr. M. Djamil Hospital in Padang. Sampling then was carried out consecutively.

The number of samples selected was twenty-five patients. Their urine was collected in the morning as much as 10 mL using a clean and dry tube. The specimens then were divided into two, one centrifuged at a rate of 1,000 g for 20 minutes, which supernatant was kept at -80°C for NAG examination, and the other centrifuged at a rate of 1,500 rpm for 10 minutes, which supernatant was stored at -20°C for albumin and creatinine examinations.

The research data were presented in the form of frequency distribution tables and diagrams. The data then were analyzed using Pearson test if normally distributed. But, if the data were not normally distributed, normalization of the data would be performed using log transformation. Next, if the data still were not normally distributed, analysis of the data would be conducted using Spearman correlation test. Results of the Spearman correlation test would indicate a good correlation if the value of r were close to 1. The data then were analyzed using a computer program.

RESULTS AND DISCUSSION

The number of the research subjects was twenty-five samples with the following basic characteristics presented in Table 1.

The research subjects were mostly dominated by males (68%). The mean age of the research subjects was 56.16±7.6 year with the age range of 39 years-67 years old. In general, the fasting blood glucose level of the research subjects were elevated, while urea and creatinine levels were still within normal limits. However, the normoalbuminuria level of those research subjects was higher (80%) than the microalbuminuria level.

The median value of UACR in this research was 6.40 (18.37) mg/g with the lowest value of 1.53 mg/g and the highest value of 119.41 mg/g. Unlike these results, a research conducted by Piwowar *et al.*[12] showed that the median value of UACR was 109 (259) mg/g in type 2 DM patients. A research conducted by Kim *et al.*[11] even also revealed that the median value of UACR was 17.43 (52.92) mg/g in patients with type 2 diabetes.

UACR value is actually determined by urine albumin and creatinine levels.[13] Urine albumin is affected by urine production determined by fluid intake. Urine production can be measured by calculating Glomerular Filtration Rate (GFR). Glomerular filtration rate is a feature of kidney filtration capacity.[14]

Nevertheless, glomerular filtration rate cannot be calculated directly. Inulin clearance is a gold standard in determining GFR. This examination, however, requires intravenous infusion and 24-hour urine collection, which takes a longer time and is considered not practical. An alternative measurement then is developed for GFR estimation using a formula. The calculation standard in estimating GFR can be based on Modification of Diet in Renal Disease (MDRD) calculation or Cockcroft-Gault equation.[14] Therefore, the differences in UACR values in this research might be due to different albumin excretions caused by

Table 1. Basic characteristics of the research subjects

	n (%)	Mean (SD)
Sex		
Males	17 (68)	
Females	8 (32)	
Age (years)		56.16 (7.6)
Fasting blood glucose (mg/dL)		148 (49)
Urea (mg/dL)		24 (6.2)
Creatinine (md/dL)		0.9 (0.3)
Urine albumin to creatinine ratio		
Normoalbuminuria	20 (80)	
Microalbuminuria	5 (20)	

* SD (standard deviation)

Table 2. Values of urine albumin to creatinine ratio and urine N-Acetyl-Beta-D-Glucosaminidase level

Variables	Median (IQR)*	Mean (SD)	The lowest level	The highest level
UACR (mg/g)	6.40 (18.37)		1.53	119.41
NAG (ng/mL)		51.01 (31.88)	9.45	144.38

* IQR (interquartile range)

GFR effects. However, this research did not aim to determine GFR values in the research subjects.

Creatinine, moreover, is produced constantly from the skeletal muscle and then excreted into the urine to maintain the normal level of creatinine in the blood. Urine creatinine excretion is affected by age, sex and weight reflecting different muscle mass.[15] Muscle mass can be measured through dual-energy X-Ray absorptiometry, bioelectrical impedance and arm circumference measurement that have a strong correlation with 24-Hour urine creatinine excretion.[14,15]

Furthermore, a research conducted by Grechman *et al.*[16] in the United States explained that body mass index (BMI) had a correlation with increased creatinine clearance. BMI measurement actually reflects the measurement of fat mass and non-fat mass relative to body weight. Increased BMI, consequently, reflects an increase in fat mass indicating obesity in a person. Obesity triggers an increase in both plasma flow to the kidneys and perfusion of the nephrons, resulting in hyperfiltration.

Hyperfiltration then stimulates the kidneys to reduce blood flow to the glomerulus by lowering GFR, leading to a reduction in both urinary creatinine excretion and creatinine clearance.[16] Nevertheless, this research did not measure BMI of each research subject. Thus, the different median values of UACR in this research from those in the previous researches conducted by Piwowar *et al.*[12] and Kim *et al.*[11] may be related to differences in GFR and BMI of the research subjects.

In addition, the mean level of NAG in type 2 DM patients in this research was considered to be high, 51.01±31.88 ng/mL since based on a research conducted by Assal *et al.*[17] on patients with type 2 diabetes in Egypt, NAG with the cut off of 15.5 ng/mL has a sensitivity of 83.8% and a specificity of 77.85% in predicting microalbuminuria in type 2 DM patients. Similarly, Ambade *et al.*[18] found an increase in NAG levels in 49.5% of patients with type 2 diabetes mellitus. Like Ambade *et al.*, Vlatkovic *et al*[7] also stated that level of NAG in type 2 diabetes mellitus patients with normoalbuminuria increased, compared to in healthy controls. The increased NAG level in microalbuminuria even is as much as three times, while in macroalbuminuria the increased NAG level is twice compared to the healthy controls. The increased NAG level indicated an increase in lysosomal activities representing an increase in proximal tubular activities in compensating for an increase in albumin filtration.[19,20] Proximal tubular activities, according to Gowda *et al.*[21] could be assessed by measuring levels of sodium, phosphate, calcium, bicarbonate, glucose, amino acids and protein in urine. A number of markers, such as beta2-microglobulin and NAG, could also be used to assess proximal tubular function.[22]

Table 3. Urine albumin to creatinine ratio and urine N-Acetyl-Beta-D-Glucosaminidase activities based on albumin excretion

Variables	Mean (SD)
UACR (mg/g)	
Normoalbuminuria	7.39 (6.59)
Microalbuminuria	86.80 (36.25)
NAG (ng/mL)	
Normoalbuminuria	37.15 (30.4)
Microalbuminuria	94.51 (65.80)

The mean value of UACR in patients with type 2 diabetes in this research was 7.39±6.59 mg/g for normoalbuminuria and 86.80±36.25 mg/g for microalbuminuria. Unlike these results, a research conducted by Assal *et al.*[17] found that the mean value of UACR in patients with type 2 DM was 13.03±8 mg/g for normoalbuminuria and 120.3±67 mg/g for microalbuminuria.

On the other hand, the mean level of NAG in type 2 diabetes patients in this research was 37.15±30.4 ng/mL for normoalbuminuria and 94.51±65.80 ng/mL for microalbuminuria. Unlike these results, the research conducted by Assal *et al.*[17] showed that the mean level of NAG was 11.2±4.5 ng/mL for normoalbuminuria and 18.5±3.7 ng/mL for microalbuminuria.

These differences in the mean values of UACR and NAG levels were due to the characteristics of the research subjects, influenced also by albumin filtration

and tubular reabsorption capacity. Album filtration was affected by glomerular damage caused by chronic hyperglycemia. Most of the research subjects in this research experienced hyperglycemia. This glomerular damage induced by hyperglycemia then would affect albumin urinary filtration.[23]

Hong & Chia[1] stated that NAG level was very easily disturbed primarily by albumin filtration. Increased albumin filtration will be reabsorbed by proximal tubules, thus increasing lysosomal activities as well as elevating NAG exocytosis to the urine. Increased NAG in urine in type 2 diabetes mellitus patients with normoalbuminuria then could indicate the presence of glomerular damage when tubular reabsorption capacity was still good.

In other words, the mean value of UACR in type 2 diabetes patients with normoalbuminuria was smaller than that with microalbuminuria. Similarly, the mean level of NAG in type 2 diabetes patients with normoalbuminuria was smaller than that in microalbuminuria.

Table 4. Correlation of urine N-Acetate-Beta-D-Glucosaminidase activity with urine albumin creatinine tatio in type 2 diabetes mellitus

Variables	NAG
UACR	r=0.614
	P<0.05

In addition, results of this research showed a strong and statistically significant correlation between urine NAG and UACR (r=0.614) (p<0.05). Similarly, a research conducted by Bouvet *et al.*[10] also revealed a strong and statistically significant correlation between urine NAG and UACR (r=0.628) (p<0.001) in type 2 DM patients. Unlike the research conducted by Bouvet *et al.*, a research conducted by Kim *et al.*[11] showed a moderate and statistically significant correlation between urine NAG and UACR (R=0.458) (p<0.001) in patients with type 2 DM.

These different results are generally affected by the characteristics of research subjects. In other words, differences in age, sex, and body weight can greatly affect the excretion of creatinine, influencing the values of UACR and NAG activities. Diabetes duration in type 2 diabetes patients as well as hyperglycemia condition can also influence UACR values.

In short, the results of this research and several previous researches have shown an increase in tubular activities due to glomerular damage in type 2 DM patients indicated by an increase in NAG. Consequently, NAG together with UACR can be used to detect glomerular and tubular dysfunctions. NAG examination is also expected to be used as a supporting test in establishing the diagnosis of early diabetes nephropathy.

However, this research still has some limitations. Firstly, the research subjects in this research were

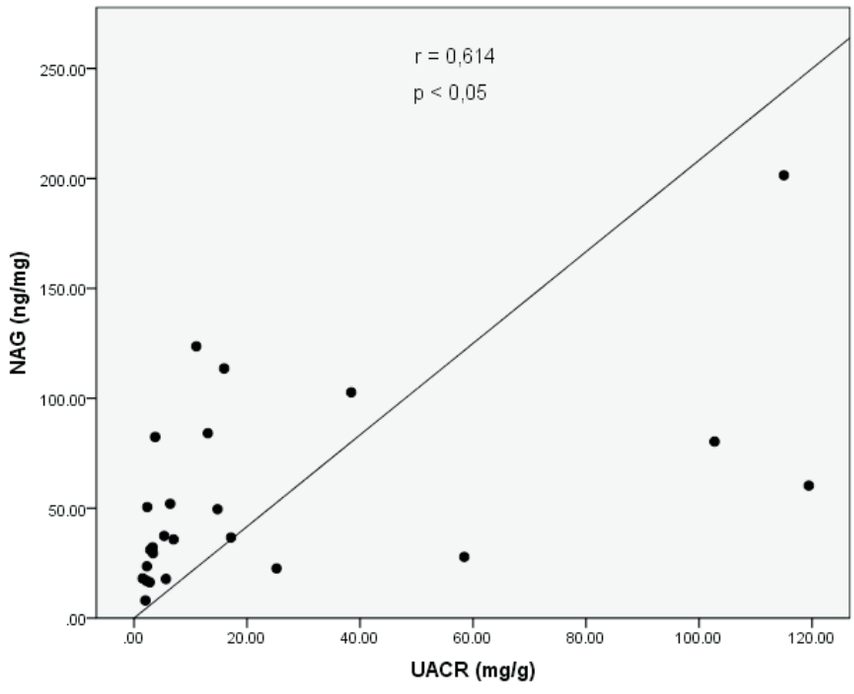

Figure 1. Correlation curve of urine NAG with UACR

selected without determining diabetes duration as well as measuring BMI, GFR, and proximal tubular function. Secondly, NAG examination was performed using an unrecognized method, so it could not compare the mean of NAG activities in this research to those in other researches using the recommended method (fluorometry) or the widely used method (spectrophotometry).

CONCLUSION AND SUGGESTION

Finally, it can be concluded that there was a strong correlation between NAG and UACR in type 2 diabetes mellitus. In this research, activities of both NAG and UACR even were proven to increase. Nevertheless, further researches on newly diagnosed type 2 DM subjects using a recommended screening method are still needed. Determination of diabetes duration, GFR, BMI and proximal tubular function are necessary to reduce factors affecting activities of UACR and NAG.

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