Differences in AGEs-N-Carboxymethyllysine and Kidney Injury Molecule-1 in non-diabetic subjects, diabetic with and without diabetic nephropathy

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ABSTRACT

Background: Diabetic nephropathy (ND) is a complication of diabetes mellitus (DM), characterized by persistent albuminuria. N-carboxymethyl lysine (CML) is the most extensive advanced glycation end products (AGEs), formed from the fructoselysine amadori. Kidney Injury Molecule 1 (KIM-1) is a type 1 transmembrane glycoprotein. The aim of the study is to analyze the differences in AGEs-CML and KIM-1 levels in the non-DM subject, DM without and with DN.

Methods: A cross-sectional analytic observational study was conducted on 25 non-DM subjects (K1), 25 DM without DN (K2), and 25 DM with DN (K3) in PROLANIS Semarang. AGEs-CML and KIM-1 levels were measured using the ELISA method. Inter-group AGEs-CML levels were analyzed using the One way ANOVA test, followed by post hoc Games-Howell. The levels KIM-1 between groups were analyzed using the Kruskal-Wallis test levels, followed by Mann Whitney post hoc test and $p<0.05$, were considered significant.

Results: There were differences in AGEs-CML levels between K1 (739.89 ± 227.37 ng/ml) and K3 (911.79 ± 107.44) ($p = 0.005$), between K2 (798.82 ± 153.03) and K3 (911.79 ± 107.44) ($p = 0.012$) and there was no difference in K1 and K2 ($p = 0.535$). There were differences in KIM-1 level between K1 [9.82 ($5.99 - 14.83$) pg/ml] and K2 [15.31 ($10.12 - 30.21$) ($p < 0.001$)], between K1 [9.82 ($5.99 - 14.83$)] and K3 [15.11 ($8.27 - 25.63$) ($p < 0.001$)] and there was no difference between K2 and K3 ($p=0.720$).

Conclusion: The highest AGEs-CML levels were significantly found in the K3 group, followed by K2 and the lowest in K3. KIM-1 levels were significantly found in the K2 group, followed by K3 and the lowest in K1.

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disease characterized by hyperglycemia due to insulin secretion abnormalities, insulin action or both.[1,2] Indonesia ranks sixth in the highest number of DM patients with 10.3 million sufferers.[3] The productive age of Indonesian people with DM that will develop kidney complications is around 35-54 years[4], while the age prevalence of type 2 DM patients with diabetic nephropathy (ND) in the United States is at the average age of 65 years.[5] Diabetes mellitus that is not managed correctly can cause chronic complications of microangiopathy and macroangiopathy.[6] Diabetic nephropathy (ND), a complication of DM microangiopathy, is the leading cause of end-stage renal disease (ESDR).[7]

Diabetic nephropathy is characterized by persistent albuminuria at least two examinations within 3-6 months, accompanied by a decrease in glomerular filtration rate.[7] Early diagnosis of ND is essential in order to get prompt treatment to inhibit the progression of ND and prevent complications.[8] Urine albumin is the gold standard parameter in diagnosing ND by measuring the level of the ratio of urine albumin-creatinine / urine albumin to creatinine ratio (uACR).[9]

However, recent opinion is that ND can occur when urine albumin levels are within the normal range. The Third National Health and Nutrition Examination Survey (NHANES III) said that about 36% of type 2 DM patients experienced a decrease in glomerular filtration rate (GFR) below 60 mL/min without changing the condition from normoalbuminuria to macroalbuminuria. Then, ND management is not optimal if it only detects albuminuria to monitor the incidence and development of ND.[10]

There are new markers that can detect early nephropathy when the patient's condition is still normoalbumin. Markers of glomerular damage include transferrin, and ceruloplasmin Type IV collagens. Markers of tubular damage include neutrophil gelatinase-associated lipocalin, kidney injury molecule-1, cystatin C, and liver-type fatty acid-binding protein. Other markers are vascular endothelial growth factor and advanced glycation end products.[11,12]

Table 1. General characteristics of research subjects

Variable	K1		K2		K3		p
	Mean±SD	(n)%	Mean±SD	(n)%	Mean±SD	(n)%	
Sex							
Male		5 (20)		6 (24)		8 (32)	
Woman		20 (80)		19 (76)		17 (68)	
Age (year)	40.64 ± 12.58		58.28 ± 5.96		55.96 ± 6.73		<0.001‡*
FPG (mg/dL)	92.84 ± 6.60		151.36 ± 69.83		164.44 ± 49.47		
Systole (mmHg)	115.40 ± 6.44		117.6 ± 5.97		118.40 ± 4.73		0.059‡
Diastole (mmHg)	74.40 ± 5.50		74.28 ± 5.83		74.60 ± 6.43		0.325‡
Urine Albumin (mg/g)	4.77 ± 2.10		10.27 ± 5.56		122.65 ± 102.20		

K1: non-DM, K2: DM without ND, K3: DM with ND, FPG: fasting plasma glucose
* Significant (p<0.05); ‡ Kruskal-Wallis

Table 2. Differences in AGEs-CML and KIM-1 in non-DM, DM without and with diabetic nephropathy subjects

Group	N	AGEs-CML Mean±SD ng/ml	p	Levene	KIM-1 Median (min-max) pg/ml	p
K1	25	739.89 ± 227.37	0.002 *⁄	0.001	9.82 (5.99 – 14.83)	<0.001*¢
K2	25	798.82 ± 153.03			15.31 (10.12 – 30.21)	
K3	25	911.79 ± 107.44			15.11 (8.27 – 25.63)	

AGEs-CML: Advanced glycation end product-N-Carboxymethyllysine, KIM-1: Kidney injury molecul-1, K1: non-DM, K2: DM without ND, K3: DM with ND
*Significant (p<0,05), ⁄One-way ANOVA, ¢Kruskall-Wallis

Table 3. Differences in levels of AGEs-CML and KIM-1 in non-DM, DM without and with diabetic nephropathy after post hoc

Group	N	AGES-CML Mean ±SD ng/ml	p	KIM-1 Median (min-max) pg/ml	p
K1	25	739.89 ± 227.37	0.535§	9.82 (5.99 – 14.83)	<0.001*†
K2	25	798.82 ± 153.03		15.31 (10.12 – 30.21)	
K1	25	739.89 ± 227.37	0.005 *§	9.82 (5.99 – 14.83)	<0.001*†
K3	25	911.79 ± 107.44		15,11 (8.27 – 25.63)	
K2	25	798.82 ± 153.03	0.012 *§	15.31 (10,12 – 30.21)	0.720†
K3	25	911.79 ± 107.44		15.11 (8.27 – 25.63)	

AGEs-CML: Advanced glycation end product-N-Carboxymethyllysine, KIM-1: Kidney injury molecul-1, K1: non-DM, K2: DM without ND, K3: DM with ND
*Significant (p<0,05), §Games-Howell post-hoc, † Mann-Whitney post-hoc.

Increased levels of advanced glycation end products (AGEs) due to prolonged hyperglycemia conditions and an increase in kidney injury molecule-I (KIM-1) in the urine can be one of the parameters for tubular damage and can be an early marker of diabetic nephropathy.[12]

Advanced glycation end products (AGEs) are a group of oxidized compounds.[13] N-carboxymethyl lysine (CML) is one of the largest components of AGEs formed from the amadori fructoselysine component formed from glucose-lysine bonds oxidative pathways.[14] High levels of AGEs can trigger oxidative stress and lead to glomerulosclerosis, interstitial fibrosis and tubulopathy which are the causes of ND.[15,16] AGEs can be a marker of early ND because there is an increase in the normoalbuminuria in DM patients.[11] Increased levels of AGEs in the urine are associated with increased markers of tubular damage in type 2 DM patients.[17] The elevated levels of AGEs reflect the onset of vascular complications.

Kidney Injury Molecule 1 (KIM-1) is a type 1 transmembrane glycoprotein. When a kidney is injured, KIM-1 is released from the cell surface into the extracellular space and appears in the urine. Damage that occurs in tubular cells will express and secrete KIM-1. This situation is an opportunity for KIM-1 to become a potential parameter for monitoring the level of inflammation in the renal tubule.[18]

Both urinary AGEs-CML and urinary KIM-1 markers can be potential early ND because the tests are both non-invasive and detectable in a normoalbuminuria patient so that if detected early, they can be managed optimally in order to prevent more progressive kidney damage.

This research aims to find out whether there were differences in levels of AGEs-CML and KIM-1 in non-DM patients, type 2 DM patients with and without early diabetic nephropathy.

METHOD

This type of research is analytic observational with cross sectional approach. This research is conducted in a chronic disease management program (PROLANIS) located at the Karang Ayu Health Center, Semarang City. Examination of AGEs-CML and KIM-

1 held in the GAKI laboratory, Faculty of Medicine, Diponegoro University, Semarang. Urine ACR examination at CITO Laboratory Semarang.

The research sample is type 2 diabetes mellitus patients without and with diabetic nephropathy at the Karang Ayu Community Health Center, Semarang City and non-DM individuals who are members of the Clinical Pathology academic community Faculty of Medicine, Diponegoro University Semarang who meet the inclusion and exclusion criteria. The research sample is divided into 3 groups, namely individuals without diabetes (K1), DM individuals without diabetic nephropathy (K2) and DM individuals with diabetic nephropathy (K3).

The research subject is taken by consecutive sampling, where every sample present at the time of data collection if they meet the inclusion and exclusion criteria is included as a sample. Samples are taken until meeting the 25 samples for each group. Total there are 75 samples.

Data analysis includes descriptive analysis and hypothesis testing. Multivariate analysis is carried out on each variable to determine the characteristics of the sample. Data for each variable is tested for data normality with the Saphiro-Wilk test, because the number of samples is less than 50. AGEs-CML data is normally distributed. One-way ANOVA statistical analysis is performed, which results are significant. The Levene test is performed to test the variance of the data, and the results are different variant data, followed by the post-hoc Games-Howell test. The KIM-1 data is not normally distributed, the data is transformed before the difference test is performed. The KIM-1 data is not normally distributed after the transformation is carried out, then it is analyzed by the Kruskal Wallis test, the results are significant followed by the post-hoc Mann Whitney test. Significance is stated at p-value <0.05.

RESULTS

There are 25 respondents for each group consisting of non-DM (K1), DM without ND (K2) and DM with ND (K3).

Subjects K1, K2, and K3 with the proportion of sex are more at the women

than men. The mean age of the subjects was older in the K2 and K3 groups than in K1.

Fasting plasma glucose on K3 has a higher mean than K1 and K2. Blood pressure in all three study groups was within normal ranges. Urinary albumin K1 levels have a mean value of 4.77 mg/g indicating normal levels. Urine albumin on K2 has a mean value of 10.27 mg/g including DM with normoalbuminuria (<30 mg/g creatinine), and urine albumin on K3 has a mean value of 122.65 mg/g including DM with microalbuminuria (30-300 mg/gr creatinine) (Table 1).

The results of data on AGEs-CML levels at K1, K2 and K3 after statistical tests are carried out with the One-way Anova test obtained significant results (p=0.002). A Levene test is performed with different data variants, then a post-hoc Games-Howell test is performed. Data are presented in the form of mean ± standard deviation (SD) (Table 2).

Data on KIM-1 levels at K1, K2 and K3 after statistical tests are carried out with the Kruskal-Wallis test obtained significant results (p<0.001). Followed by the post-hoc Mann-Whitney test. Data are presented in median form (min-max) (Table 2).

AGEs-CML levels on K1 has a mean value of 739.89 ± 227.37 ng/ml, K2 has a mean value of 798.82 ± 153.03 ng/ml and K3 has a mean value of 911.79 ± 107.44 ng/ml (Table 3).

There is a significant difference in AGEs-CML levels between the K1, K2 and K3 groups with p = 0.002. AGEs-CML levels are almost the same in K1 and K2. There is no significant difference in AGEs-CML levels between K1 and K2 (p = 0.535). AGEs-CML levels in K3 are higher than K1. There is a significant difference in AGEs-CML levels in K1 and K3 (p=0.005). AGEs-CML levels in K3 are higher than K2. There is a significant difference in AGEs-CML levels at K2 and K3 (p=0.012) (Table 3).

KIM-1 levels in K2 and K3 are higher than K1. There are significant differences in KIM-1 levels at K1, K2 and K3 with p <0.001. KIM-1 levels in K2 are higher than K1. There is a significant difference in KIM-1 levels in K1 and K2 (p<0.001). KIM-1 levels in K3 are higher than K1.

There is a significant difference in KIM-1 levels in patients K1 and K3 (p<0.001). KIM-1 levels in K2 are no different than K3. There is no difference in KIM-1 levels in K2 and K3 (p=0.720) (Table 3).

DISCUSSION

The percentage of female patients in this study is greater than the men. Research by Gale and Gillespie in 2001 stated that the incidence of type 2 diabetes in the age range of 25 - 44 years in women increased by about 20%, ages 35 - 44 years to 60%, age 45 to 64 years the ratio of women is twice that of men.[19]

The K1 study subjects have a younger age compared to DM patients, which have a mean age of 40 years. The mean value of K2 and K3 ages are around 55-58 years. Research results stated that individuals aged $\geq$ 45 years are more at risk of developing DM than individuals aged <45 years.[20] The proportion of people with diabetes increases with age.

Urine albumin on K1 has a mean of 4.77 mg/g, K2 has a mean albumin level of 10.27 mg/g according to the theory, it is included in the normoalbuminuria group (ACR level <30 mg/g) and K3 has a mean ACR level of 122.65 mg/g, entered into the microalbuminuria group (30-300 mg/g). Urine albumin as an early predictor of ND complications in DM patients is considered the best predictor of kidney damage in helping early diagnosis of ND.[21]

The mean value of GDP levels at K1 is 92.84 mg/dl (normal reference <110 mg/dl). The mean values of GDP K2 are 151.36 mg/dl and K3 is 164.44 mg/dl, indicating that the patient has diabetes mellitus. This shows that poorly controlled basal blood sugar levels can increase risk factors for diabetic nephropathy.[22] Microalbuminuria contributes to the development of insulin resistance and type 2 diabetes.[23]

Hyperglycemia triggers oxidative stress and inflammation, which contribute to diabetic nephropathy. Increased expression of AGEs increases oxidative stress in cells causing an inflammatory response. The accumulation of AGEs in the kidneys contributes to changes in kidney structure and loss of kidney function. AGEs formation also contributes to GBM thickening and mesangial expansion which are characteristic features of ND.[24]

Significant differences in AGEs-CML levels are found in K1, K2 and K3 with a value of p=0.002. The increase in CML excretion is thought to be due to the start of the tissue glycoxidation process and kidney damage.[25,26]

There is no significant difference in levels of AGEs-CML in K1 and K2 with a value of p=0.535. Urinary CML levels do not increase in DM patients who have not experienced renal impairment, the levels were the same as normal controls.[27]

AGEs-CML levels in K1 are lower than K3 with a significant difference with a value of p=0.005. The renal tubule is a potential target for CML, especially in diabetic patients. The formation of AGEs-CML plays a central role in the development of tubular dysfunction in diabetic nephropathy, which activates intracellular signals triggering free oxygen radicals. AGEs receptors (RAGEs) can induce pTEC activation and tubular dysfunction. The excretion of pTEC plays an essential role in renal tubular damage.[28]

AGEs-CML levels in K2 are lower than K3 with a significant difference with a value of p=0.012. There is an increase in urinary CML excretion in type 1 DM patients with micro and macroalbuminuria compared with patients who were in normoalbuminuria.[25]

There is a significant difference in KIM-1 levels at K1, K2 and K3 with a p-value <0.001. The highest mean KIM-1 levels are in the DM group without and with ND (microalbuminuria). Kidney injury molecule-1 is not secreted under normal conditions, so it is a sensitive marker when kidney damage begins, and the levels will return to normal when the epithelial tissue begins to repair.[29,30]

KIM-1 on K1 is lower than that in K2 with a significant difference in KIM-1 levels in K1 and K2 with p-value <0.001. KIM-1 levels can increase when the kidneys are injured/ischemic to the proximal tubular epithelium.[18] This shows that KIM-1 can be used as an early biomarker of renal tubular damage in DM patients.[31]

KIM-1 levels in K1 are lower than those in K3 with significant differences with p value<0.001. KIM-1 is expressed in the apical membrane of proximal tubular cells. KIM-1 will appear in the urine if there is an ischemic state. This marker will

not appear if the kidneys are in normal condition, therefore KIM-1 can be used as a specific and sensitive marker for the proximal tubule.[32]

KIM-1 levels in K2 are slightly higher than those in K3. There is no significant difference in KIM-1 levels in K2 and K3 with p value=0.720. Urine KIM-1 is higher in diabetes mellitus patients with normoalbuminuria than normal controls and there is no much difference from DM patients who experienced albuminuria. Increased urine KIM-1 may represent subclinical disorders characterized by the appearance of tubular markers in the urine before the occurrence of kidney damage.[18] Urine KIM-1 has increased in DM with normoalbuminuria and CKD stage 2 but falls again in CKD stages 3 and 4, this is due to atrophy and fibrosis in the tubules so that KIM-1 levels have decreased along with worsening kidney conditions.[33]

This study does not consider the types of high-CML foods and the high temperatures foods process that may affect urine AGEs-CML levels. Screening for exclusion criteria for history of urinary tract infection, which could affect KIM-1 levels, was performed using only a questionnaire. Different age ranges in the non-DM group and type 2 DM patients are thought to affect KIM-1 levels.

CONCLUSION

There is no difference in AGEs-CML in non-DM subjects with DM without diabetic nephropathy. There is a significant difference in AGEs-CML in non-DM subjects with diabetic nephropathy DM. There is significant difference in AGEs-CML in DM without and with diabetic nephropathy. There is a significant difference in KIM-1 in non-DM subjects with DM without diabetic nephropathy. There is significant difference in KIM-1 in non-diabetic subjects with diabetic nephropathy DM. There is no difference in KIM-1 in DM without and with diabetic nephropathy.

ETHICAL APPROVAL

The study has received ethical clearance approval from the Health Research Ethics Commission of the Faculty of Medicine, Diponegoro University, Semarang No.

20/EC/KEPK/FK-UNDIP/II/2020. All research subjects are asked for consent with written informed consent before the study is conducted. The identity of the patient will be kept confidential and all costs associated with this study will be the responsibility of the investigator.

AUTHOR CONTRIBUTION

Dwi Fajaryani responsible for writing the original draft and project administration Muji Rahayu and Banundari Rachmawati supervision of the project, main idea, and writing the original draft. All author had reviewed and agreed for the final version of the article.

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