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"Correlation between Serum Glycated Albumin and Malondialdehyde with Urinary Nephrin in Type 2 Diabetic Mellitus Patients"

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CORRELATION BETWEEN SERUM GLYCATED ALBUMIN AND MALONDIALDEHYDE WITH URINARY NEPHRIN IN TYPE 2 DIABETIC MELLITUS PATIENTS

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ABSTRACT

Background: Type 2 diabetes mellitus (T2DM) is one of the global health problems with increasing prevalence and with large medical and social impacts. T2DM can cause micro and macrovascular complications because of chronic hyperglycemia and blood glucose levels fluctuations. Both of these components lead to the occurrence of T2DM complications through two main mechanisms, which are excessive glycosylation of proteins and oxidative stress. The process of glycosylation and oxidative stress can damage several tissues, such as kidney, heart, and vascular. Damage in kidney tissues can occur in several histologic structures, which is podocyte. Glycated albumin levels describe the tissue glycosylation, malondialdehyde levels describe the presence of oxidative stress, and urinary nephrin levels describe kidney podocytes damage. It is important to understand the correlation between serum glycated albumin and malondialdehyde with urinary nephrin in type 2 diabetes mellitus patients. The aim of this study is to describe the correlation between serum glycated albumin and malondialdehyde with urinary nephrin in T2DM patients.

Methods: This is an observational research with cross sectional method. The sample was 30 patients with T2DM that controlled to outpatient department in Dr. M. Djamil Padang Hospital. Serum glycated albumin levels was measured by enzimatic colorimetric method, serum malondialdehyde levels was measured by Buege method and urinary nephrin levels was measured by ELISA.

Results: Of the 30 patients with T2DM, there was a significant increase in glycated albumin and malondialdehyde levels. The mean level of serum glycated albumin was 20.87 (5.91%) and the median level of serum malondialdehyde was 9.24 nmol/ml. The median level of nephrin urine was 369.25 ng/ml. There was a significant positive correlation between serum glycated albumin and serum malondialdehyde levels with urine nephrin levels with correlation coefficient respectively $r = 0.404$ and $r = 0.528$.

Conclusion: Tissue glycosylation and oxidative stress process have a role in podocytes damage in T2DM patients. Increased serum malondialdehyde levels has more effect than serum glycated albumin levels in its effect on urinary nephrin levels in T2DM patients.

Keyword: T2DM, glycated albumin, malondialdehyde, nephrin.

Introduction

Diabetes mellitus (DM) is one of the world's health problem with increasing prevalence and important medical and social impact. According to the International Diabetes Federation (2013), 382 million people across the world had DM in 2013. The global prevalence of diabetes in 2035 is predicted to increase to 592 million people. In 2013, Southeast Asia holds 72 million adults with diabetes and is estimated to increase to 123 million people in 2035. A significant increase on the DM prevalence occurs in Southeast Asia including Indonesia. In 2030 DM sufferers is estimated to be 21,3 million people.[1,2]

Diabetes mellitus can cause microvascular complication, including diabetic kidney disease (PGD).[3,4] Based on the American Diabetes Association (ADA), PGD occurs in 20-40% DM patient and becomes the main cause of end stage renal disease.[5,6]

Protein in human body can be glycated. Glycation of various proteins increases in diabetic patients, as of these glycated proteins can be utilized to evaluate diabetes controlling. Of all glycated proteins, HbA1c is the gold standard for glycaemic control. In the last few years glycated albumin (GA) becomes an index of glycation for medium range uncontrolled blood glucose because of the short albumin half-life compared to erythrocyte.[7,8]

Oxidative stress is caused by unbalanced production of oxidant or ROS and detoxification capacity to repair cell damage. There is a direct relationship between kidney damage severity with oxidative stress level in diabetic kidney disease. Several markers has been used to measure oxidative stress in diabetic patients. One of the marker is malondialdehyde (MDA).[9,10]

In DM patient, a further pathologic kidney change occurs. There are three components that becomes the glomerular filtration barrier, which is the podocyte, capillary endothelial cell, and glomerular basal membrane. Certain proteins can reflect the condition of podocyte and nephrin. In diabetic condition, a downregulation of nephrin take place and acts as an antiapoptotic agent. Released nephrin escapes through the urine and can be detected in patients urine. The loss of nephrin cause flattening foot process of the podocyte and cause the increasing of proteinuria.[11,12]

Many studies shows that hyperglycaemia play role in the pathogenesis of diabetic kidney disease, so that a glycaemic control to prevent complication is needed. Complication in diabetic patients is caused by the protein glycation process and the oxidative stress occurred in patient. Based on those backgrounds, we decided to study the correlation between uncontrolled blood glucose with urinary nephrin level in patient with diabetes mellitus type 2.[11,12]

Method

This study is a cross sectional observation study. Study is conducted in the inpatient room and polyclinic of the Internal Medicine Department RSUP M. Djamil Padang for 6 months. The population of this study is patients with type 2 DM, hospitalized in RSUP M. Djamil Padang or routine polyclinic patients within the age of 18-59 years. Samples are population that match the inclusion and exclusion criteria.

A correlation analysis made between serum glycated albumin with urinary nephrin level and the correlation between serum malondialdehyde level and urinary nephrin level. Data is processed with SPSS 21.0, value of significance is calculated, and is significant if $p<0,05$.

Results

Table 1 shows the characteristics of 30 type 2 DM patients. This study consists 11 male patients (36,67%) and 19 females (63,3%). The mean age of the type 2 DM patients is 52,03 years old, the youngest is 37 years old and the oldest is 59 years old. The most amount of age obtained are 51-59 years old. Patients distribution based on the age groups are, 30-39 years old is 1 patients (3,33%), the groups of 40-49 years old has 8 patients (26,67%) and the age 50-59 years old as much as 21 patients (70%). The mean duration of disease suffering is 7,3 (5,4) years. The mean body mass index is 21,67 (3,9) kg/m^2. In this study, the average fasting blood glucose is 178,8 (71,4) mg/dl and post prandial blood glucose is 219,1 (89,7) mg/dl. Mean ureum level is 34,7 (29,4) mg/dl and creatinine is 1,1 (0,5) mg/dl.

Table 1. Baseline Characteristics

Charateristics	**n (%)**	**Mean (SD)**
Gender		
Male	11 (36,67)	
Female	19 (63,33)	
Age (year)		52,03 (5,60)
30 - 39	1 (3,33)	
40 – 49	8 (26,67)	
50 – 59	21 (70)	
Duration of disease suffering (year)		7,30 (5,40)
Body Mass Index (kg/m^2)		21,67 (3,90)
Fasting Blood Glucose (mg/dl)		178,80 (71,40)
Post Prandial Blood Glucose (mg/dl)		219,10 (89,70)
Ureum (mg/dl)		34,70 (29,40)
Creatinine (mg/dl)		1,10 (0,50)

In this study we obtained mean level of serum glycated albumin at 20,87 (5,91) % (normal value 11-16 %). Result of Kolmogorov Smirnov normality test shows the serum glycated albumin in this study is normally distributed. After performing one sample t test, we found the GA level increases significantly with p<0,001.

Table 2. Serum Glycated Albumin in Type 2 DM patients

Variable	**n**	**Mean (SD)**
***Glycated Albumin* (%)**	30	20,87 (5,91)

glycated albumin normal value: 11- 16%

In this study we obtained the level of serum malondialdehyde as much as 9,24 nmol/ml, with the lowest level 5,59 nmol/ml and the highest is 13,56 nmol/ml. Kolmogorov Smirnov normality test shows the data of serum malondialdehyde level in the study is not normally distributed. Table 3 shows the median level of serum malondialdehyde.

Table 3. Serum Malondialdehyde Level in Type 2 DM patients

Variable	n	Median	Minimum – Maximum
Serum Malondialdehyde (nmol/ml)	30	9,24	5,59 – 13,56

Serum MDA normal value: 0,9 - 1,59 nmol/ml

In this study, the median level of urinary nephrin is 369,25 ng/ml, with the lowest level is 6 ng/ml and the highest is 3952 ng/ml. Kolmogorov Smirnov normality test shows the data of urinary nephrin in the study is not normally distributed. Table 4 provides the median urinary nephrin level.

Tabel 4. Urinary Nephrin Level in Type 2 DM Patients

Variable	n	Median	Minimum – Maximum
Urinary Nephrin (ng/ml)	30	369,25	6 – 3952

Urinary nephrin normal value: not detected

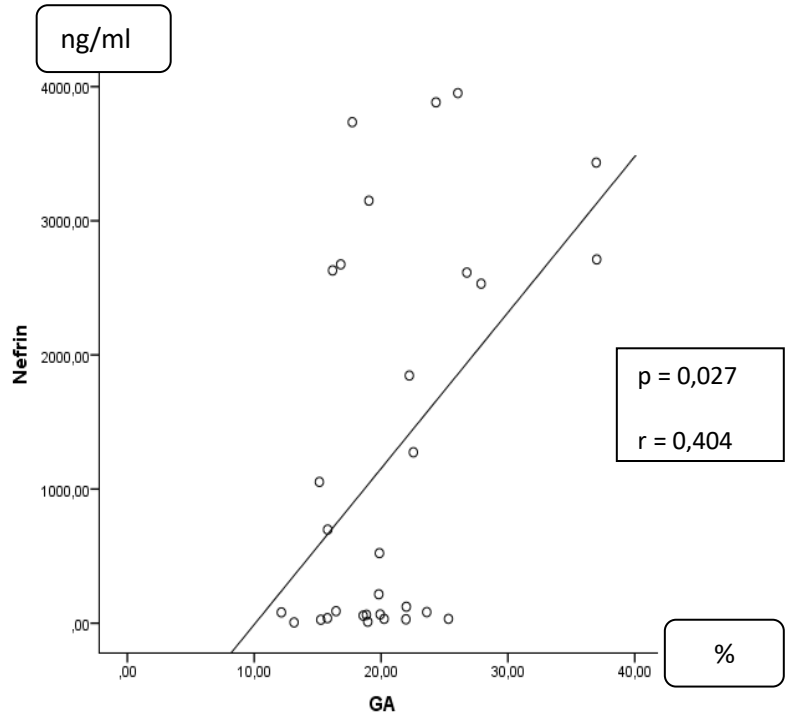

Figure 1. Graphic of correlation between serum glycated albumin level with urinary nephrin level in the Type 2 DM patients

In figure 1 above, can be seen the correlation between serum glycated albumin level with urinary nephrin level in the Type 2 DM patients. The correlation analysis

used is the Spearman correlation test and confidence level obtained is p<0,05. The analysis result shows a significant correlation between serum glycated albumin with urinary nephrin level (p=0,027), with positive correlation and the correlation strength is moderate. (Correlation coefficient r=0,404)

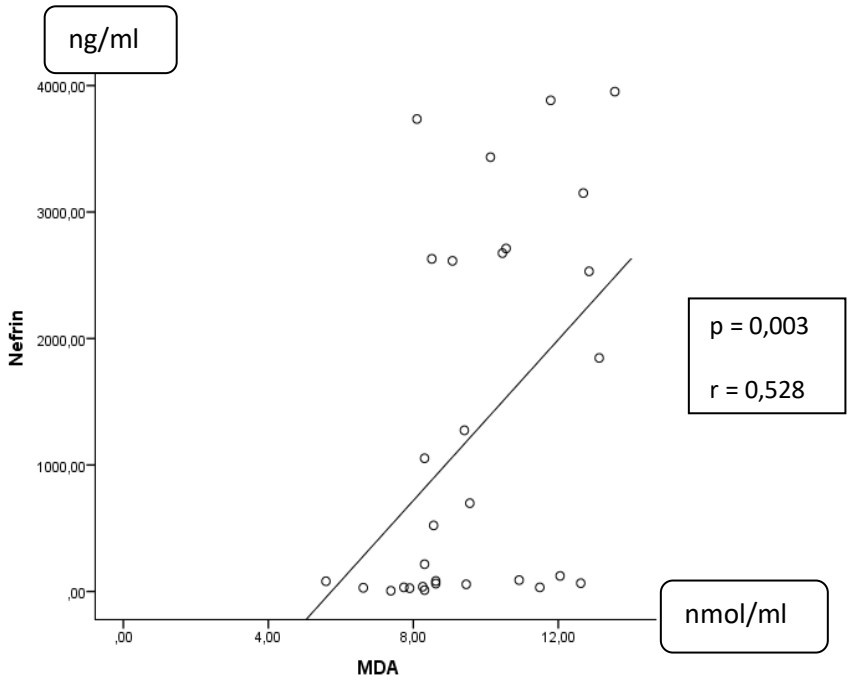

Figure 2. Correlation graphic between serum malondialdehyde level with urinary nephrin level in type 2 DM patients.

In the figure above can be seen the correlation between serum malondialdehyde level with urinary nephrin level in type 2 DM patients. Correlation analysis used is Spearman correlation test and confidence level obtained is p<0,05. The analysis result shows a significant correlation between serum malondialdehyde level with urinary nephrin level (p=0,003) with positive direction and moderate strength of correlation (correlation coefficient r=0,528)

Discussion

From 30 type 2 DM samples, 19 patients (63,33%) were female and 11 are male (36,67%). Study by Furusyo *et al* (2011) on DM population in Japan found that there are more female patients than male with a ratio of 70:30. Hilawe *et al* (2013) published a meta-analysis of 36 study and states that the prevalence of type 2 DM in South Africa is much higher in women compared to men.[13,14]

In this study we found the mean age of type 2 DM patients treated in RSUP Dr. M. Djamil Padang is 52,03 (5,6) years old with the most amount of age groups are 50-59 years old, which is 21 patients (70%). Lopez *et al* (2014) performed a study in New Jersey with 7,239 type 2 DM patients and found the mean age is 59,9 years old. Whereas Solini *et al* (2013) reported a multicenter study of type 2 DM in Italia and found an older age range compared to the study, which is 59-73 years old.[15,16]

In this study the mean disease suffering of the type 2 DM patients is 7,3 (5,4) years. There is 1 patient who just noticed that he already suffered type 2 DM after 1 month. Study by Petrica *et al* (2014) in Greece found that the mean duration of disease suffering in type 2 DM patients is 9 years. Wang *et al* (2016) in his study of DM population in China found the mean duration of disease suffering is 9 (3) years. While Solini *et al* (2013) found the mean duration of disease suffering in Italia is more longer, which is 11 years.[16,17,18]

Mean body mass index (BMI) in this study is 21,67 (3,9) kg/m^2. A similar result shown by Furusyo *et al* (2011) in Japan DM population, who found a mean BMI of 21,6 kg/m^2. Xu Y *et al* (2013) studied the type 2 DM population in China and obtained a mean BMI 23,7 kg/m^2, while Flegal KM *et al* (2010) found a mean BMI he surveyed as 28,7 kg/m^2 in the population of America.[13,19,20]

Spearman test result between serum GA and urinary nephrin in this study reveals a statistically significant result (p=0,027) with a positive direction and moderate strength correlation (correlation coefficient r=0,404). Correlation between serum GA and urinary nephrin shows a positive correlation, means that the higher the serum GA level, the higher the level of urinary nephrin. In this study we identify that uncontrolled blood glucose shown by GA level will aggravate the diabetic kidney disease, which is reflected by the urinary nephrin.[21]

Study of Doublier in 2003 was the first study that correlates glycated albumin and nephrin expression. This study investigates the nephrin distribution in kidney biopsies of 17 patients with DM and nephrotic syndrome, DM microalbuminuria and 10 control subjects. To the samples, GA was also examined. To determine whether the nephrin expression is influenced by GA, the podocyte was incubated with GA for 48 hours. The study demonstrated a result that GA induced a reduction of nephrin expression on the podocyte surface.[22]

Study by Cohen *et al* (2013) measured the level of glycated albumin, creatinine, albumin and nephrin in diabetic murines compared to nondiabetic murines. This study demonstrated a positive linear correlation between GA and urinary nephrin ($p<0,05$ and r=0,58). An increase in serum GA concentration is followed by nephrin level increase in diabetic murines. Conversely, if serum GA level is reduced by controlling hyperglycaemia, the urinary nephrin also decreased.[23]

The correlation strength between serum GA level and urinary nephrin level in this study shows a moderate correlation (r=0,404). We presume that the presence of genetic factor affects the diabetic kidney disease. Chen G *et al* (2013) performed a study in Japan to identify genetic factors predisposing type 2 DM and the gene related to diabetic complications, such as cardiovascular risks and diabetic kidney disease. This study identifies a presence of 17 gene related to diabetes and 4 gene related to diabetic kidney disease. Patients with those genes will promote diabetic kidney disease faster than DM patients without these genes.[24]

Bandeira *et al* (2013) stated that DM complications can occur as a result of excess oxidative stress and protein glycation. Protein glycation, including albumin, will produce AGEs. Complications will happen if AGEs bounds to its receptor (RAGE). If AGEs doesn't bound to RAGE, then there will be no induction towards cytokines production and oxidative stress, therefore complications will not appear. Aside from the binding between AGEs and RAGE, DM complications can also happen due to increased intracellular glucose that activates the oxidative stress pathway. In this

study, it is not certain whether AGEs and RAGE binding or oxidative stress directly to the cell caused diabetic nephropathy.[4]

A Spearman test between serum MDA level and urinary nephrin shows a statistically significant result (p=0,003) with a positive correlation and moderate strength (correlation coefficient r=0,528). Correlation between serum MDA and urinary nephrin shows a positive correlation, that means the higher the level of serum MDA, the higher urinary nephrin. This study presented that oxidative stress described by MDA level will aggravate the diabetic kidney disease, which is defined by urinary nephrin.[21]

We have not found any former research associated to the relation between serum MDA and urinary nephrin in type 2 DM patients. Li *et al* (2010) investigated the relation between oxidative stress and nephrin using protein oxidative stress marker, the *advanced oxidation protein products* (AOPPs). This study reveals an exposure of AOPPs towards podocyte will induce a decrease in nephrin expression.[25]

The correlation strength of serum MDA level with urinary nephrin level in this study demonstrated a moderate correlation (r=0,528). Genetic factor influences the incident of diabetic kidney disease. Gu *et al* (2013) proposed a relation between genetic polymorphism of ICAM1 (*Intercellular Adhesion Molecule 1*) with diabetes and diabetic kidney disease. The limitation of this study is the research did not reach those genetic aspects.[26]

CONCLUSION

Tissue glycosylation and oxidative stress process have a role in podocytes damage in T2DM patients. Increased serum malondialdehyde levels has more effect than serum glycated albumin levels in its effect on urinary nephrin levels in T2DM patients.

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