ANALYSIS OF URINE MATRIX METALLOPROTEINASE-9 IN DIABETIC NEPHROPATHY

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Abstract

Matrix metalloproteinase-9 is a protease enzyme that plays a role in tissue destruction, tissue remodelling, and inflammation and is a proteinase studied in human PGD. Protein MMP-9 concentration and activity are increased in the urine of T2DM patients, most common in patients with albuminuria, and correlate with kidney injury. This study aims to determine and compare the MMP-9 levels in urine in diabetic and non-diabetic nephropathy subjects. The study design was cross-sectional with a total sample of 52 samples; there were 26 samples from diabetic nephropathy and 26 from non-diabetic nephropathy. MMP-9 urine levels were examined using the ELISA (*Assay Genie, Irelaind*) method. The results showed that there was a significant difference between urinary MMP-9 in diabetic nephropathy compared to DM without nephropathy (14.04 ng/mL and 3.07 ng/mL). Correlation test results of urine MMP-9 and urine albumin were r =0.53, p<0.001 (p≤0.05), and the AUC value obtained from the ROC method is 83.1%(95% CI 71.5% - 94.7%), p = <0.001 from a cut-off value of 3.96 ng/mL with a sensitivity and specificity value of 76.9% in diagnosing diabetic nephropathy. The conclusion is that urine MMP-9 levels are higher in diabetic nephropathy subjects than DM without nephropathy; there is a significant correlation between urine albumin levels and urine MMP-9 in DM subjects, and the higher the urine albumin level, the higher the urine MMP-9 levels.

Keywords: Type 2 Diabetes Mellitus,MMP-9, Diabetic Nephropathy

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Introduction

Diabetes mellitus is a Non-Communicable Disease (PTM) with a relatively high mortality rate of around 2.2 million and will continue to increase until 2035 to around 600 million people(1). The International Diabetes Federation (IDF) states that in 2013–2017 there will be an increase in the number of DM sufferers from 10.7 million to 16.7 million by 2045, while in 2021 worldwide, there will be around 537 million cases of DM aged 20 - 79 years (1 in 10people) suffers from DM(2). Indonesia ranks 5th with DM sufferers of 19.47 million out of a population of around179.72 million, with a DM prevalence of 10.6% (3).

Diabetic nephropathy is a kidney function disorder caused by DMT2, which can affect the ability of the kidneys to excrete fluids and toxins from the body. Early symptoms of diabetic kidney disease are characterised by changes in the glomerular basement membrane and mesangial expansion.

Matrix metalloproteinases (MMP) are endopeptidases that collectively degrade all components of the extracellular matrix (ECM) content and basement membrane protein sthatplayarolein controlling the pathophysiology of tissuere modelling and maintenance, renal tissue, and regulate chemokine release. Increased concentration of MMP-9 levels in urine is a sign of diabetic nephropathy, kidney disorders and urinary tract infections(UTI) (4).

Suggested that urinary MMP-9 activity in diabetic nephropathy increases albuminuria and correlates with signs of diabetic nephropathy, urinary MMP activity is a sensitive, noninvasive, and clinically useful biomarker for predicting vascular remodeling in diabetic renal and vascular complications (5).

Materials and Methods

Location and Design Study

Sampling was conducted at the Endocrinology Polyclinic at Hasanuddin University Hospitaland the Clinical Pathology Laboratory at Hasanuddin University Hospital to recruit research subjects. The research was carried out at the Hasanuddin University Medical Research Center (HUM-RC) Laboratory, Hasanuddin University State University Hospital, Makassar. The research design uses observational analytic with a cross sectional approach.

Population and Research Sample

The study population consisted of all subjects, both patients diagnosed with diabetic nephropathy aged >18 years. The sample in this study was 52 samples using a non-probability sampling technique, namely purposive sampling, which complied with the inclusion criteria, and were willing to participate in this study and signed informed consent.

Instrument and data collection

The research sample used a urine specimen, namely mid-urine (the first and last urine were not collected, only midstream urine was collected). The collected urine was divided into two, onepart for quantitative examination of urine albumin levels using the Cobas C311 analyser using the immunoturbidimetric assay method. One more portion was stored at -20°c (for 2-3 months),then followed by an examination of urine MMP-9 levels using the Enzyme-linked immunosorbent assays (ELISA), by the Sandwich method using the Assaygenie test kit reagent (Europe (Ireland) which has a sensitivity <0.188 ng/mL and a limit value range of 0.313 – 20 ng/mL. Specimens were analys edaccording to the test kit instructions.

Data Analysis

Data analysis was performed using a statistical computer program (SPSS). Data analysis is univariate and bivariate analysis, with unpaired t-tests. The normality test (Shapiro-Wilk test) was used because the variable urine albumin was not normally distributed, and the comparison test (Mann-Whitney test) was because the urine MMP-9 variable was not normally distributed. The test results are significant if$p \leq 0.05$.

Ethics

The research was conducted after obtaining ethical approval from the Hasanuddin University Research Ethics Commission and permission for sampling from the Hasanuddin State University Hospital (UHS Hospital), with ethical number 640/UN4.6.4.5.31/PP36/202

Results

The sample used in this study was 52 samples; there were 26 diabetic nephropathies and 26 non-diabetic nephropathies. The distribution of research subjects is shown in table1.

Table 1 General Distribution of Research Subjects Based on Gender, Age and Urinary Albumin.

Frequency (person)		n	%
Sex			
Male		25	48,1
Female		27	51,9
Total		**52**	**100**
Age	30-40 years	3	5,8
	41-50 years	5	9,6
	51-60 years	20	38,5
	61-70 years	20	38,5
	>70 years	4	7,7
Total		**52**	**100**
Urinary Albumin Levels	< 30 mg/dL	26	50
	≥ 30 mg/dL	26	50
Total		**52**	**100**

Source: Primary Data

Table 1 shows the distribution of research subjects in general based on the sex characteristics of 25 males (48.1%) and 27 respondents (51.9%) females based on the age of the research subjects the most age is 51-60 and 61 -70 years each, 20 respondents (38.5%), ages41-50 years five respondents (9.6%), ages > 70 years four respondents (7.7%), and the minor age is 30-40 years with a total of 3 respondents (5.8%), based on urine albumin levels <30mg/dLand urine albumin levels≥30 mg/dLas many as26 respondent seach (50.0%).

Table 2. Comparison in Urine MMP-9 levels in Diabetic Nephropathy and Non-Diabetic Nephropathy Subjects

Variable	Condition	Mean	SD	p-value
MMP-9Urine	Diabetic Nephropathy	14,04	20,69	<0,001
(ng/mL)	Non-Diabetic Nephropathy	3,07	2,84	

*Uji *Mann-Whitney Test*

Table 2 shows the average urine MMP-9 level in diabetic nephropathy of 14.04 ng/mLwith a standard deviation of 20.69 ng/mL. Incomparison, the average urine MMP-9 level in non-diabetic nephropathy subjectsis3.07 ng/mL, with a standard deviation of 2.84 ng/mL. This shows a significant difference between urine MMP-9 in diabetic nephropathy subjects compared to non-diabeticpatients

Table3. Correlation of Urine MMP-9with Urinary Albumin

Relation	r	p-value
MMP-9 Urine dan Albumine Urine	0,53	<0,001

**Rank Spearman Test Correlation Test*

Table 3 shows the correlation test results between urine MMP-9 and urine albumin using the Spearman rank correlation test (r=0.53 and p= <0.001). This shows a moderate correlation and shows that there is a significant relationship between MMP- 9 urine with urine albumin.

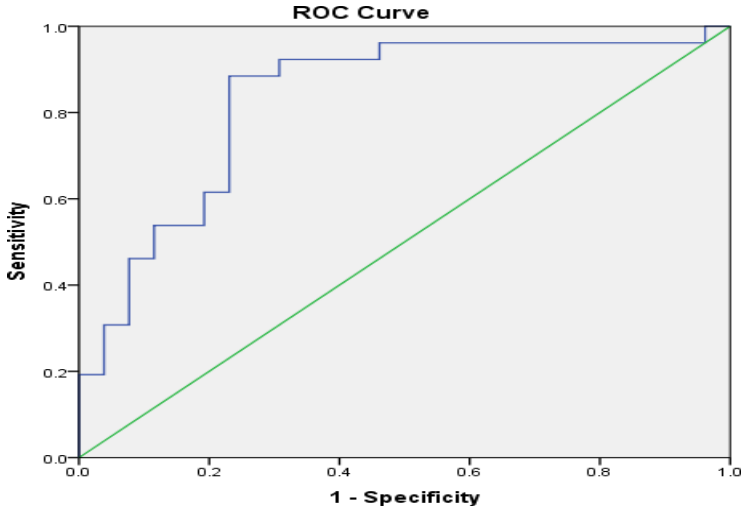

Figure1.MMP-9 Urine ROC Curvein Diagnosing Diabetic Nephropathy

Table5. Test results Area Under the Curve (AUC) for urine MMP-9 variables

Area	*Std.Error*[a]	*AsymptoticSig.*[b]	*Asymptotic95%Confidence Interval*	
			Lower limit	**Upper Limit**
0,83	0,06	<0,001	0,72	0,95

Nullhypothesis = 0.5

Figure 1 shows 26 of 52 subjects with nephropathy based on the Case Processing Summary, with a prevalence of diabetic nephropathy cases of 50%. At the output, there is aROC curve indicating that urine MMP-9 has good diagnostic value because the curve is far from the 50% line and close to 100%. The AUC value obtained from the ROC method was83.1% (95% CI 71.5% - 94.7%), p = <0.001 based on table 5. Statistically, the AUC value of83.1% was classified as vital. MMP-9 urine level of 3.96 ng/mL has a sensitivity value of 76.9% and as pecificity of 76.9%.

Discussion

From the results of this study, there appeared to be a significant difference in the average urine MMP-9 levels (p<0.001) in the diabetic nephropathy group (14.04 ± 20.69 ng/mL) higher than in the non-diabetic group. Diabetic nephropathy (3.07±2.84 ng/mL).

Based on the ROC value, the AUC value was 83.1%,and the MMP-9 urine cut-off value was ≥3.96, with a sensitivity and specificity value of 76.9%.

Albumin in kidney parietal epithelial cells shows signs of MMP-9 excretion in the urin. Matrix Metalloproteinase-9 is a Zn+2-dependent endopeptidase in the form of a protein encoded by the MMP-9 gene, produced by macrophages and granulocytes in the form of MMP-9. Normal physiological processes such as embryonic development, reproduction, tissue remodeling, endothelial damage, and angiogenesis will have implications for the development of vascular disease and malignancy (6).

In a study that examined the correlation between serum MMP-9 levels and albumin uria levels in T2DM patients, the results stated that there were a significant relationship between serum MMP-9 levels and urine albumin-creatinine ratio (ACR) levels in DM subjects (r=0.064, p=0.0001) (7).

A study in Mexico also reported that urine albumin levels were higher in study subjects with renal impairment and MMP-9 levels were higher in the female group than in the male group(8).

In this study, in line with a study which showed that urinary MMP-9 activity in diabetic nephropathy appears early be for eurinary albumin, urinary MMP activity increases in the adolescent age group in the early stages of T2DM (3).

This study is also in line with studies on urinary MMP levels in children with chronic kidney disease, which stated that urine MMP-9 levels were significantly increased in CKD patients compared to the control group and increase durinary MMP-9 level score lated significantly with serum values (9).

This study is different from a study conducted by Alan Uriel García-Tejeda et al. (2018) in Mexico which reported that there was no relationship between urine MMP-9 levels and age, body mass index (BMI), waist circumference, number of drugs taken for treatment T2DM and fasting glucose levels (8).

Systemichy pertension can cause hyperfiltration and hemodynamic abnormalities, leading to glomerular damage and diabetic nephropathy. Abnormal intraglomerular hemodynamic processes will change the growth and function of glomeruli, mesangial and epithelial cells by increasing physical and mechanical stress, which will trigger the formation of increased mesangial matrix and thickening of the basement membrane, which is characteristic of diabetic nephropathy. Diabetic nephropathy is a disease complication characterised by aprogressive decline in kidney function and systemic abnormalities such a sproteinuria, hypertension, and other signs of chronicrenal insufficiency (10).

Chronic hyperglycemia activates NF-κB, which can trigger the expression of various cytokines, chemokines, cell adhesion molecules, and MMP-9. Tumour necrosis factor (TNF-α) can be regulated by NF-κB and is also a potent activator of NF-κB, induces insulin resistance mainly through serine phosphorylation of insulin receptor substrates and can trigger the formation of oxidative stress which can lead to increased activity MMP-9 (11,12).

The increase in urine MMP-9 levels in diabetic nephropathy subjects reported in this study indicates the involvement of MMP-9 in the process of nephropathy due to DM (13).This research is cross-sectional, so it cannot explain the causality between urine albumin and MMP-9.

Conclusions

The results of a comparison test for urine MMP-9 levels in nephropathy subjects werehigher than DM without nephropathy. This shows that there is a significant difference between urinary MMP-9 in diabetic nephropathy compared to non-diabetic nephropathy. In future studies, it is hoped that research on urine MMP-9 levels in advanced diabetic nephropathy can be carried out.

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