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ANALYSIS OF URINE MINDIN TYPE 2 DIABETES MELLITUS PATIENTS

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Abstract

Type 2 diabetes mellitus is not only caused by an inadequate insulin secretion, but also from the impaired responsiveness or failure of insulin-target cells to appropriately react to insulin. Mindin is a secreted extracellular matrix (ECM) protein, exerting a broad spectrum of effects on the innate immune system. Mindin is correlated with diabetic nephropathy, podocyte injury, colitis, allergic asthma, liver ischemia and reperfusion injury, as well as ischemic brain injury. This study aims to determine urinary mindin levels in patients with type 2 diabetes mellitus and non-diabetes mellitus. Cross sectional analytic observational study with a total of 76 subjects consisting of 51 T2DM Subjects and 25 non-DM. Mindin levels were measured by ELISA method and ACR by Immunoturbidimetry. The results showed a comparison test obtained a p-value of 0,128, which is greater than 0,05 (p>0,05). This indicates that the value of urine mindin in Type 2 DM and non-DM research subjects has no significantly different influence.

Keywords: Mindin Urine, Type 2 Diabetes Mellitus.

INTRODUCTION

The World Health Organization (WHO) in 2010 suggested that 60% of all-age deaths in the world are caused by non-communicable diseases (NCDs). Diabetes mellitus (DM) is the sixth leading cause of death. About 1,3 million people die from diabetes mellitus and 4% die before the age of 70. It is estimated that by 2030, diabetes mellitus will be the seventh leading cause of death in the world. In general, low-income countries show the lowest prevalence of diabetes while middle-income countries show the highest prevalence of diabetes in the world WHO, 2015.

The prevalence of diabetes in the world is estimated at 2,8% in 2020 and will increase to 4,4% in 2030. The number of people with diabetes is projected to increase from 171 million in 2020 to 366 million in 2030. Asian countries account for >60% of the diabetes population worldwide [1]. According to the International Diabetes Federation (IDF), the prevalence of diabetes mellitus in the world is 1,9% and makes diabetes mellitus as the seventh leading cause of death worldwide, while in 2012, the number of cases of diabetes mellitus in the world was 371 million people, with a proportion of cases amounting to 95% of the world's population experiencing diabetes mellitus [2].

In Indonesia, the prevalence of people over 15 years of age with diabetes mellitus in 2013 was 6,9% with an estimated number of cases of 13.191.564 million. The 30,4%

of cases were previously diagnosed and 73,7% were undiagnosed. Indonesia with a population of approximately 210 million people, 10 million people suffer from diabetes mellitus. This makes Indonesia becomes the fourth country with the most cases of diabetes after India, China, and the United States [3].

Type 2 diabetes mellitus is a metabolic disorder characterized by an increase in blood glucose due to decreased insulin secretion by pancreatic beta cells or impaired insulin function or referred to as insulin resistance [4]. Type 2 diabetes mellitus is not only caused by a lack of insulin secretion, but also because insulin target cells fail or cannot respond to insulin normally [5].

Mindin (spondin2), a secretory protein associated with neurodevelopment and immunity, is a member of the *thrombospondin type I repeat* (TSR) superfamily of proteins, and has a unique glycosylation *C-mannosylation* in its structure. It remains unclear whether *C-mannosylation* plays a functional role in mindin biosynthesis in cells, and *C-mannosylation* plays a functional role in the anchorage of the superfamilic protein TSR in cells [6]. Mindin is a secreted *extracelullar matrix* (ECM) protein, exerting a broad spectrum of effects on the innate immune system [7].

Mindin is associated with diabetic nephropathy, podocyte injury, colitis, allergic asthma, liver ischemia and reperfusion injury, as well as ischemic brain injury. On the other hand, it is reported as a protective factor against obesity, cardiac hypertrophy, and fibrosis [8].

MATERIALS AND METHODS

Method and Location

This research was conducted with observational analytic method using cross sectional study design. This study was conducted to analyze urine mindin in patients with type 2 diabetes mellitus. Sampling process was done at Endocrine Poly, Clinical Pathology Laboratory, Hasanuddin University Hospital Makassar. Urine Mindin examination was conducted in the research unit, Hasanuddin University Hospital Makassar.

Population and Sample

The target population of this study was adult patients with type 2 diabetes mellitus both outpatients and inpatients who fit the criteria and control non-DM people. The samples in this study were samples taken from a population of adult subjects suffering from type 2 diabetes mellitus and non-DM subjects as controls.

The study sample was a sample that met the inclusion criteria with the sample size calculated as follows:

$$n1 = n1 = \frac{[(Z\alpha + Z\beta)S\ ]^2}{x1\text{-}x2}$$

n1 = minimum sample size of group 1

n2 = minimum sample size of group 2

Zα = standardized alpha derivative, calculated from the type I error. In this study, the type I error was set at 5% so the Zα value was 1,645.

Zβ = standardized beta derivative, calculated from type II error. In this study, the type II error was set at 20%, so the value of Zα was 0,842.

S = the standard deviation, according to Sopiyudin 2013, the standard deviation is obtained if there is no similar research by multiplying the minimum average difference by 2, so the researchers get the value of s is 8.

X1-X2 = the minimum mean difference that is considered meaningful by the researchers. In this study, the meaningful value set was 4.

$$n1 = n2 = \left[\frac{(1.645 + 0.842)8}{4} \right]^2$$

$$= \left[\frac{(2,487)\ 8}{4} \right]^2$$

$$= \left[\frac{19.896}{4} \right]^2$$

$$= 4,974^2$$

= 24.74 rounded to 25.

Based on the calculation with the formula above, the minimum sample is 25 samples for the T2DM group and 25 samples for the non-diabetes group with a total of 50 people.

Sampling Method

The sampling technique in this study is non-probabilty purposive sampling, which is a sample determination technique by selecting samples among the population according to the the researcher, so that the sample can represent the characteristics of the population that have been previously known. The sample criteria in this study include:

1. Inclusion Criteria
    a. Adult patients with type 2 diabetes mellitus
    b. Respondents who are willing to be sampled in the study and are willing to sign an informed consent.
2. Exclusion Criteria
    a. Pregnant or breastfeeding women
    b. Acute and chronic renal failure
    c. Other chronic diseases
    d. Actively consuming alcohol

Data Analysis

After the data was collected, it was processed and presented in the form of frequency distribution tables, then analyzed. Data processing was carried out using statistical software and analyzed by bivariate analysis.

RESULTS

Table 1 shows the characteristics of respondents of type 2 DM research, it is known that the most genders as the objects are female as many as 28 respondents (54,9%)

and male as many as 23 respondents (45,1%), the largest age distribution is age 51 - 60 years as many as 20 respondents (39,2%), age 28 - 40 years and > 70 years have the same number of respondents as many as 3 respondents (5,9%), age 41 - 50 years as many as 6 respondents (11,8%), 61 - 70 years as many as 19 respondents (37,3%), while albumin creatinine levels are the most common. The 70 years had the same number of respondents as 3 respondents (5,9%), 41 - 50 years of age as many as 6 respondents (11,8%), 61 - 70 years as many as 19 respondents (37,3%), while albumin creatinine ratio levels in type 2 DM subjects whose levels are <30 mg/g of 32 respondents (62,7%) and levels ≥30 mg/g are 19 respondents (37,3%), HbA1c levels in type 2 DM subjects whose levels are <7% of 9 respondents (17,6%), and levels ≥7% are 42 respondents (82,4%).

Table 1: Characteristics of Research Respondents of Type 2 DM Subjects

Characteristics of Type 2 DM	Frequency (n=51)
Gender	
Male	23 (45,1)
Female	28 (54,9)
Age	
28 - 40 years	3 (5,9)
41 - 50 years	6 (11,8)
51 - 60 years	20 (39,2)
61 - 70 years	19 (27,3)
> 70 years	3 (5,9)
Albumin Creatinine Ratio	
< 30 mg/g	32 (62,7)
≥ 30 mg/g	19 (37,3)
HbA1c	
< 7%	9 (17,6)
≥ 7%	42 (82,4)
Source: Primary Data	

Table 2 shows the characteristics of Non-DM research respondents, it is known that the most gender is female as many as 15 respondents (60.0%) and male as many as 10 respondents (40,0%), the most age distribution is age 28-40 years as many as 20 respondents (80,0%), age 41-50 years as many as 4 respondents (16,0%), and age 51-60 years as many as one respondent (4,0%).

Table 2: Characteristics of Non-DM Subjects

Characteristics of Type 2 DM	Frequency (n=51)
Gender	
Male	10 (40,0)
Female	15 (60,0)
Age	
28 - 40 years	20 (80,0)
41 - 50 years	4 (16,0)
51 - 60 years	1 (4,0)
Source: Primary Data	

Based on Table 3, it shows the average comparison of urine mindin levels in Type 2 DM and Non DM subjects, the average urine mindin value in Type 2 DM subjects is 41,20 pg/mL with a standard deviation of 309,13 pg/mL, while the urine mindin value in non-DM subjects is 33,00 pg/mL with a standard deviation of 378,56 pg/mL. This shows that the average value of urine mindin in type 2 DM research subjects is higher than that of non-DM.

Table 3: Comparison Test of Urinary Mindin Levels in Type 2 DM and Non DM Research Subjects

	Conditions	N	Mean	Std. deviation	p-value*
Mindin (pg/mL)	Type 2 DM	51	41,20	309,13	0,128
	Non-DM	25	33,00	378,56	

**Mann Whitney Test*

In this study, the results of the comparison test obtained a p-value of 0,128, which is greater than 0,05 (p>0,05). This indicates that the urine mindin value in Type 2 DM and non-DM research subjects is not significantly different.

DISCUSSION

Mindin is a secreted protein that promotes adhesion and growth of hippocampal embryonic neurons in vitro. Mindin is reported to function as an integrin ligand [9]. Integrins are also key molecules for podocyte injury. Based on research [8]. Suggested that mindin is associated with podocyte injury and diabetic nephropathy. In immunohistochemical staining, mindin protein is localized in podocytes because its expression from WT1 is limited to podocytes in adult glomeruli [10].

Podocytes cover the outer aspect of the glomerular basement membrane (GBM) and form the final barrier to protein loss [11]. Podocyte foot processes are fixed to the GBM via integrin α3β1 and α/β dystroglycans, and integrin α3β1 is the major integrin expressed by podocytes [12]; [13]. Moreover, Jia et al. also reported that mindin functions as an integrin ligand. Their research also found that β1 integrin protein expression was increased in cultured podocytes stimulated under HG conditions [9]. Mindin is produced by damaged podocytes under high glucose conditions and serves as a biomarker of the progression of diabetic nephropathy.

Based on Table 4. The comparison test results obtained a p-value of 0,128, which is greater than 0,05 (p>0,05). This indicates that the value of urine mindin in Type 2 DM and non-DM research subjects is not significantly different [14]. Which suggests that the decreased podocyte density and increased mindin expression observed in all cases of diabetic nephroathy, indicating that mindin expression is significantly higher. In addition, mindin protein showed high specificity in biopsies of patients with diabetic nephropathy (p<0,0001). The study conducted by Martin, suggests that mindin protein may play a role in the pathogenesis of diabetic nephropathy and is a promising biomarker for detecting podocyte lesions.

While the study by Kahvecioglu et al 2015 suggested that there were no significant differences between groups in terms of demographic data, C-reactive protein, HbA1c, lipids, serum uric acid levels and leukocyte counts. Mindin levels were observed to increase linearly with increasing severity of diabetic nephropathy. The mindin levels in Group 4 (albuminuria and serum creatinine >1.5 mg/dL) were significantly higher than Group 1 (normoalbuminuria) and the control group, and the mindin levels in Group 3 (macroalbuminuria) were also significantly higher than Group 1 (normoalbuminuria). Blood urea nitrogen, creatinine, and urinary albumin excretion rate (UAER) were significantly positively correlated with mindin; serum total protein, and calcium levels were negatively correlated with mindin in DN patients. The researchers conclude that there is a linear and significant increase in mindin levels for patients with type 2 DM as the stage of diabetic nephropathy increases, but serum mindin levels are not as

effective as microalbuminuria in reflecting nephropathy. In addition, serum mindin levels are not as good as urine and tissue mindin levels in detecting renal damage in diabetic nephropathy [15].

Increased podocyte foot processes were observed in rats fed a *high-caloie* diet (HC). The expression level of mindin protein in rats was localized in podocytes, and its level in the glomerulus was increased in the HC group compared to the standard diet (SD) group. Urinary mindin levels in the HC group at 16 weeks of age were also significantly higher compared to the SD group although the *albumin creatinine ratio* (ACR) did not differ between the two groups. In addition, mindin levels in patients with type 2 DM were higher than those in healthy individuals and increased gradually as ACR increased. In conclusion, mindin may be associated with podocyte injury and appears to be an early biomarker of the development of diabetic nephropathy [10].

Increasing evidence suggests that inflammation and immune response mechanisms may contribute significantly to the development and progression of diabetic nephropathy. Recent studies indicates that mindin plays a crucial role for the initiation of immune response and serves as a pattern recognition molecule in the ECM. Studies have suggested that mindin could potentially serve as an early biomarker for the progression of diabetic nephropaty [16].

CONCLUSION

The findings of the study suggest that, om average, the levels of urine mindin in individuals with type 3 diabetes mellitus (DM) are higher compared to those without DM. However, there appears to be no significant distinction in urine mindin levels between individuals with type 2 DM and those without the condition.

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