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

PAGE 185
2022 March; 28(2): 185-190
p-ISSN 0854-4263 e-ISSN 2477-4685
Available at www.indonesianjournalofclinicalpathology.org

Analysis of Neutrophil Gelatinase-Associated Lipocalin in Type 2 Diabetes Mellitus Patients

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ABSTRACT

Neutrophil Gelatinase Associated Lipocalin (NGAL) is a small protein with a molecular weight of 21 kDa, which can be found in epithelial cells of the renal tubules and is increased in the blood after injury the kidneys. Chronic hyperglycemia in uncontrolled type 2 Diabetes Mellitus (DM) can cause microvascular complications such as impaired kidney function (diabetic nephropathy). The aim of the study was to determine the NGAL levels in controlled and uncontrolled type 2 DM patients. This study was cross-sectional research involving a total of 70 type 2 DM patients consisting of 30 patients with controlled type 2 DM and 40 patients with uncontrolled type 2 DM. HbA1c, urea, and creatinine levels were measured, and a urinalysis was carried out. Neutrophil gelatinase-associated lipocalin level was measured using the ELISA method. The statistical tests used in this study were Chi-Square, unpaired T-test, Mann-Whitney test, and Spearman test significance p-value <0.05. There was a significant difference in NGAL levels between the controlled (25.09±6.83) and uncontrolled type 2 DM groups (112.54±170.38 ng/mL) with a p-value <0.001. There was a positive correlation between NGAL and HbA1c levels with r=0.507, p-value <0.001, and creatinine levels with r =0.769 and p<0.001. There was a significant difference in NGAL levels between the controlled and uncontrolled type 2 DM patients. A positive correlation in this study indicated that higher HbA1c and creatinine levels led to higher NGAL levels.

Keywords: Type 2 diabetes mellitus, NGAL

INTRODUCTION

Type 2 Diabetes Mellitus (DM), according to the American Diabetes Association (ADA) 2019, is a metabolic disease characterized by hyperglycemia that occurs due to the inability of the pancreas to secrete insulin and/or impaired insulin action. The Basic Health Research (Riskesdas) report by the Ministry of Health of the Republic of Indonesia in 2018 showed that the average prevalence of DM in the population aged 15 years was 10.9%. Indonesia has the 7th highest number of DM sufferers globally, with a total of 10.3 million diabetic patients in 2019. This number is expected to increase to 13.7 million people in 2030 and 16.6 million people in 2045.[1-4]

The pathogenesis of hyperglycemia in type 2 diabetes involves insulin resistance and impaired insulin secretion. Inflammation is a major contributing factor to insulin resistance in type 2 diabetes. Inflammation produces various proinflammatory cytokines and chemokines, such as Tumor necrosis Factor-α (TNF-α) and Interleukin-6 (IL-6), which can interfere with insulin action. Diabetes mellitus can cause various complications, including acute and chronic complications that damage small blood vessels (microvascular) and large blood vessels (macrovascular).[5-8] Chronic hyperglycemia can cause oxidative stress, inflammation, and formation of Advance Glycation End-Products (AGEs) that will cause damage to endothelial cells, mesangial cells, and renal tubular epithelial cells, leading to impaired kidney function and diabetic nephropathy.[9-11]

Neutrophil Gelatinase Associated Lipocalin (NGAL) is a small protein with a molecular weight of 21 kD, which belongs to the lipocalin protein family and functions as a growth and differentiation factor for renal tubular epithelial cells. Usually, NGAL in the circulation is filtered by the glomerulus and then reabsorbed by the proximal renal tubular epithelial cells. Increased levels of NGAL (10-fold increase) can be detected in 2-6 hours in the blood and urine after injury to the kidneys. Neutrophil gelatinase associated lipocalin in the blood indicates damage to the proximal renal tubular epithelial cells.[12-14] Several studies on NGAL, such as a study by Fodor *et al.*, and

Khawaja *et al*., found that NGAL can be used as an early marker for the Acute Kidney Injury (AKI) in critically ill patients admitted to the Intensive Care Unit (ICU).[14,15] A study by Corbacioglu *et al*. found higher NGAL levels in AKI than in Chronic Kidney Disease (CKD).[16] In addition, a study by Patel *et al*. and Seibert *et al*. found that NGAL had a prognostic value on kidney function in CKD patients.[17,18]

A study by Kim found an increase in NGAL levels in diabetic nephropathy accompanied by macroalbuminuria.[19] A study by Wu found that an increase in NGAL levels was associated with the incidence of nephropathy in type 2 DM patients.[20] A study by Li found that increased NGAL levels can be used as a marker of renal insufficiency normoalbuminuria in type 2 DM patients.[21]

Based on this background, the authors aimed to perform a study on the analysis of NGAL levels in type 2 DM patients.

METHODS

This study was an observational study with a cross-sectional method, which was carried out by collecting primary data from outpatient samples from the Metabolic Endocrine Outpatient Clinic-Internal Diseases Dr. Wahidin Sudirohusodo Hospital, Makassar and Hasanuddin University Hospital, with a diagnosis of type 2 DM between July and September 2020. Sampling procedure, urinalysis, and measurement of GDP, HbA1c, urea, and creatinine levels were carried out at the Laboratory of Clinical Pathology of Dr. Wahidin Sudirohusodo Hospital, Makassar, and Hasanuddin University Hospital. In contrast, measurement of NGAL levels was carried out at the Laboratory of Research Unit of FKUH/Hasanuddin University Hospital.

The population in this study was all adult patients who underwent examinations at the Outpatient Clinic of Internal Medicine and Metabolic Endocrine, Dr. Wahidin Sudirohusodo Hospital, Makassar, and Hasanuddin University Hospital, and those diagnosed with type 2 DM between July and September 2020. The inclusion criteria in this study were as follows: Adults the age of 18 years who were diagnosed with type 2 DM based on the ADA criteria 2019, such as fasting plasma glucose of 126 mg/dL or plasma glucose of 200 mg/dL after Oral Glucose Tolerance Test (OGTT) or plasma glucose value of 200 mg/dL with classic complaints or HbA1c value ≥ 6.5%; Patients who underwent urinalysis admeasurement of GDP, HbA1c, urea, and creatinine levels; Patients who were willing to participate in the study by signing the informed consent. Contrastingly, exclusion criteria in this study were hemolysis, icteric, lipemic samples, patients with hematological disorders (impaired erythrocyte life span), patients who had recently received whole blood or packed red cell transfusions in the last three months, patients with inflammation/infection, patients with a history of malignancy, and patients who were undergoing hemodialysis. Research subjects with HbA1c values <7% were classified into the controlled type 2 DM group, and subjects with HbA1c of 7% were classified into the uncontrolled type 2 DM group. The estimated Glomerular Filtration Rate (GFR) was calculated using the Chronic Kidney Disease-Epidemiology Collaboration (CKD-EPI) formula (mL/min/1.73m^2) as follows:

$$GFR = 141 \times \min(Scr/k,1)^{\alpha} \times \max(Scr/k,1)^{-1.209} \times 0.993^{age} \times 1.018 \text{ (if female)} \times 1.159 \text{ (if African-American)}$$

Abbreviations:
Scr : Serum creatinine in mg/dL
K : 0.7 for female and 0.9 for male
A : -0.329 for female and -0.411 for male
min : minimum Scr/k or 1
max : maximum Scr/k or 1

Fasting blood sugar (GDP) was measured using the enzymatic method (Hexokinase) and the ABX Pentra 400 analyzer, and the result was expressed in mg/dL. HbA1c levels were measured using the immunoturbidimetric method by Konelab and expressed in percent (%). Urea levels were measured using the Enzymatic UV test method and ABX Pentra 400 analyzer, and the results were expressed in mg/dL. Creatinine levels were measured using the Jaffe reaction method and ABX Pentra 400 analyzer and expressed in mg/dL. Urine analysis was carried out using the Dirui H-800 Automatic Urine Analyzer. Serum NGAL was measured using the Enzyme-Linked Immunosorbent Assay (ELISA) method (Human NGAL Elisa Kit, Bioassay Technology Laboratory) and expressed in ng/mL.

The research permission was obtained from the Health Research Ethics Commission, Faculty of Medicine, Hasanuddin University, with the number 564/UN4.6.4.5.31/PP36/2020.

Data were analyzed using SPSS version 22. The statistical tests used in this study were Chi-Square, unpaired T-test, Mann-Whitney test, Kolmogorov-Smirnov test, and Spearman test. P-value <0.05 was stated significantly.

RESULTS AND DISCUSSIONS

According to the HbA1c levels, the general characteristics of the research subjects were divided

into two groups, as controlled and uncontrolled type DM group, as shown in Table 1. Therefore, this study involved 70 research subjects with a controlled type 2 DM group consisting of 30 subjects and an uncontrolled type 2 DM group consisting of 40 subjects.

The controlled and uncontrolled type 2 DM groups in this study showed the same composition of subjects based on gender, consisting of 60% male and 40% female subjects. A study by Wu *et al*. and Li *et al*. found that the prevalence of type 2 DM sufferers was higher in males than females.[20,21] The mean age in the controlled and uncontrolled type 2 DM group in this study was 58.7±7.7 years and 57.2±10.8 years, respectively. There was a significant difference in the duration of DM between the controlled and uncontrolled type 2 DM groups (p=0.005).

Table 1. General characteristics of research subjects based on controlled and uncontrolled type 2 DM

Characteristics	Controlled Type 2 DM (n=30)	Uncontrolled Type 2 DM (n=40)	P
Gender			
Male	18 (60%)	24 (60%)	1.000*
Female	12 (40%)	16 (40%)	
Age (years)			
Mean±SD	58.7±7.7	57.2±10.8	0.527**
Duration of DM (years)			
Mean±SD	3.7±3.2	6.8±5.2	0.005***
BMI (kg/m^2)			
Mean±SD	25.5±3.4	25.9±3.2	0.582**
Blood pressure (mmHg)			
Systolic (mean±SD)	133.8±12.2	137.3±13.6	0.243***
Diastolic (mean±SD)	78.5±9.8	78.9±7.8	0.894 ***

Note: * Chi-Square test ** Unpaired T-test ***Mann-Whitney test BMI: Body Mass Index Source: Primary Data

Table 2. Results of hematological tests, blood chemistry tests, and urinalysis of controlled and uncontrolled type 2 DM group

Parameter (Mean±SD)	Controlled Type 2 DM (n=30)	Uncontrolled Type 2 DM (n=40)	P
Hemoglobin (gr/dL)	13.9±1.1	14.1±1.5	0.726*
Leukocytes (x10$^{3/}$uL)	7.98±1.61	8.04±1.48	0.875**
Platelets (x10$^{3/}$uL)	291±55	291±124	0.260*
FGL (mg/dL)	107,6±19.4	178,5±74.3	<0.001*
HbA1c (%)	6.3±0.7	8.9±1.3	<0.001*
Urea (mg/dL)	29.2±9.9	41.2±26.4	0.011*
Creatinine (mg/dL)	0.82±0.21	1.67±0.84	<0.001*
GFR	93.1±44.5	49.4±20.9	<0.001*
Urine protein			
Negative	26 (86.7%)	30 (75%)	0.974***
1+	4 (13.3%)	7 (17.5%)	
2+	0 (0%)	2 (5%)	
3+	0 (0%)	1 (2.5%)	
Urine glucose			
Negative	30 (100%)	33 (82.5%)	0.670***
1+	0 (0%)	4 (10%)	
2+	0 (0%)	1 (2.5%)	
3+	0 (0%)	2 (5%)	

Note: *Mann-Whitney test ** Unpaired T-test ***Kolmogorov-Smirnov Z test
FGL: Fasting Glucose Levels, GFR: Glomerular Filtration Rate
Source: Primary Data

Based on Table 2, there were significant differences in GDP, HbA1c, creatinine, and GFR between the controlled and the uncontrolled DM group. This was in line with research conducted by Wu *et al.* and Li *et al.*, which found higher levels of GDP, HbA1c, and creatinine but lower GFR values in type 2 DM patients.[20,21] Glycated hemoglobin or HbA1c can indicate the mean blood glucose levels in the previous 2-3 months. There was a correlation between blood glucose levels and HbA1c levels, suggesting that the amount of HbA1c glycation produced is proportional to blood glucose levels. The ADA also recommends the HbA1c test as a long-term blood glucose control, a diagnostic marker, and a predictor of the development of diabetes complications. Chronic hyperglycemia in DM patients causes the formation of non-enzymatic protein glycation processes, HbA1c, and causes microvascular complications, such as diabetic nephropathy. Hyperglycemia is recognized as a significant risk factor for diabetic nephropathy but is not responsible for all changes in renal tissue. The pathophysiology of kidney damage in type 2 DM patients is due to the interaction between hemodynamic and metabolic factors and the presence of inflammatory factors. Hyperfiltration, glomerular hypertrophy, thickening of the basement membrane, and accumulation of extracellular matrix (MES) occurs initially. However, if glycemic control cannot be controlled, glomerulosclerosis and interstitial fibrosis will occur in the kidney at an advanced stage, which can lead to proteinuria, decreased kidney function, and decreased creatinine clearance.[6-8,12-14]

From Table 3 and Figure 1, it was found that there were significant differences in the mean NGAL levels between the controlled and uncontrolled type 2 DM group, with $p < 0.001$. In a study conducted by Kim *et al.*, NGAL levels increased higher in patients with diabetic nephropathy. In addition, a study conducted by Wu *et al.* and Li *et al.* found increased NGAL levels in type DM patients.[2,19-21] Neutrophil gelatinase associated lipocalin is a small protein with a molecular weight of 21 kDa, which belongs to the lipocalin protein family. Neutrophil gelatinase associated lipocalin is present in the secondary

Table 3. Comparison of NGAL levels between controlled and uncontrolled type 2 DM group

NGAL	Controlled Type 2 DM (n=30)	Uncontrolled Type 2 DM (n=40)	p*
Mean±SD (ng/mL)	25.09±6.83	112.54±170.38	<0.001

Note: * Mann-Whitney test NGAL: Neutrophil Gelatinase-Associated Lipocalin Source: Primary data

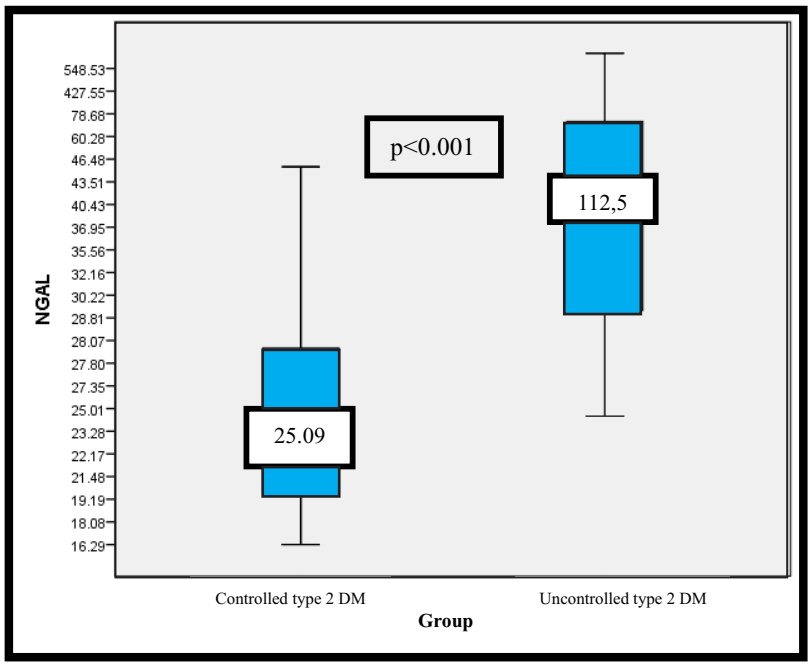

Figure 1. Neutrophil gelatinase associated lipocalin levels in controlled and uncontrolled type 2 DM group

Table 4. Correlation of NGAL with HbA1c, creatinine, and GFR in type 2 DM patients

Parameter	Mean±SD	NGAL r	p*
HbA1c (%)	7.81±1.66	0.507	<0.001
Creatinine (mg/mL)	1.30±0.77	0.769	<0.001
GFR (mL/min)	68.13±39.42	-0.710	<0.001

Note: * Spearman test NGAL: Neutrophil Gelatinase Associated Lipocalin, GFR: Glomerular Filtration Rate
Source: Primary Data

granules of neutrophils, which are released when neutrophils are activated. This protein functions as a growth and differentiation factor for various cell types, such as renal tubular epithelial cells. Usually, NGAL in the circulation is filtered by the glomerulus and then reabsorbed by the proximal renal tubular epithelial cells. Increased NGAL levels can be detected in 2-6 hours in the blood and urine after injury to the kidneys. Excretion of NGAL in the blood occurs when there is damage to the epithelial cells of the proximal renal tubules. In several studies, NGAL has been used as a predictor of impaired renal function, especially in patients with Acute Kidney Injury (AKI), because it can be detected 1-2 days before the increase of serum creatinine.[9-11,14,15]

Based on Table 4, there was a positive correlation between HbA1c and NGAL, suggesting that higher HbA1c levels led to higher NGAL levels with moderate correlation strength. In addition, there was a positive and robust correlation between NGAL and creatinine levels. In addition, there was a significant and strong correlation between NGAL and GFR ($p<0.001$), suggesting that higher NGAL levels led to a lower GFR.

CONCLUSIONS AND SUGGESTIONS

In this study, a significant difference in NGAL levels was found between the controlled and uncontrolled type 2 DM group, and a positive correlation was found between NGAL and HbA1c levels. Higher HbA1c levels led to higher NGAL levels. Increased NGAL levels in uncontrolled type 2 diabetes in this study were unable to predict impaired kidney function. Therefore, it was expected that further research on diabetic nephropathy subjects could add other kidney function parameters such as microalbumin, cystatin c, and Kidney Injury Molecule-1 (KIM-1).

REFERENCES

1. American Diabetes Association (ADA). Standards of medical care in diabetes. Diabetes Care, 2019; 42(1): S1-S2.
2. American Diabetes Association (ADA). Classification and diagnosis of Diabetes: Standards of medical care in diabetes. Diabetes Care, 2019; 42(1): S13-S28.
3. Kementerian Kesehatan Republik Indonesia. Hasil utama RISKESDAS. 2018 Available from: https://www.kemkes.go.id/resources/download/info-terkini/hasil-riskesdas-2018.pdf (accessed March 20, 2020).
4. International Diabetes Federation. Diabetes and cardiovascular disease. Brussels, International Diabetes Federation, 2019; 9: 1–176.
5. Huang D, Refaat M, Mohammedi K, Jayyousi A, Suwaidi JA, Khalil CA. Macrovascular complications in patients with diabetes and prediabetes. BioMed Research International, 2017; 1-9.
6. American Diabetes Association (ADA). Microvascular complications and foot Care: Standards of medical care in diabetes. Diabetes Care, 2019; 42(1): S124-S138.
7. Yim J, Kim G, Lee BW, Kang ES, Cha BS, *et al*. Relationship between circulating netrin-1 concentration, impaired fasting glucose, and newly diagnosed type 2 diabetes. Frontiers in Endocrinology, 2018; 9: 691-8.
8. Selby NM, Taal MW. An updated overview of diabetic nephropathy: Diagnosis, prognosis, treatment goals, and latest guidelines. Diabetes Obes Metab, 2020; 22(1): 3–15.
9. Khausik U, Lewis JB. Update on diabetic nephropathy: Core curriculum. American Journal of Kidney Disease, 2018; 71(6): 884-895.
10. Alicia RZ, Rooney MT, Tuttle KR. Diabetic kidney disease: challenges, progress, and possibilities. Glomerular disease. Clinical Journal of American Society of Nephrology, 2017; 12(12): 2032-2045.
11. Shen Z, Fang Yi, Xing T, Wang F. Diabetic nephropathy: From pathophysiology to treatment. Journal of Diabetes Research, 2017: 1-2.
12. Ning M, Mao X, Niu Y, Tang B, Shen H. Usefulness and limitations of neutrophil gelatinase-associated lipocalin in the assessment of kidney disease. Journal of Laboratory and Precision Medicine, 2018; 3: 1-10.
13. Tang XY, Zhou JB, Lou FQ, Han YP, Zhao W, Diao ZL, *et al*. Urine NGAL as an early biomarker for diabetic kidney disease: Accumulated evidence from observational studies. Renal Failure, 2019; 41(1): 446-454.
14. Khawaja S, Jafri L, Siddiqui I, Hashmi M, Ghani F. The utility of Neutrophil Gelatinase-Associated Lipocalin (NGAL) as a marker of Acute Kidney Injury (AKI) in

critically ill patients. Biomarker Research, 2019; 7(4): 1-6.
15. Fodor R, Grigorescu B, Veres M, Orkandea M, Badea J, *et al*. Plasma Neutrophil Gelatinase-Associated Lipocalin (NGAL)-early biomarker for acute kidney injury in critically ill patients. The Journal of Critical Care Medicine, 2015; 1(40): 154-161.
16. Corbacioglu SK, Cvik Y, Akinci E, Uznosmanoglu H, Dagar S, *et al*. Value of plasma neutrophil gelatinase-associated lipocalin in distinguishing between Acute Kidney Disease (AKI) and Chronic Kidney Disease (CKD). Turkish Journal of Emergency Medicine, 2017; 17: 85-88.
17. Patel ML, Sachan R, Misra R, Kamal R, Shyam R, Sachan P. Prognostic significance of urinary NGAL in chronic kidney disease. International Journal of Nephrology and Renovascular Disease, 2015; 8: 139-144.
18. Seibert FS, Sitz M, Passfall J, Hausner M, Laschinski P, *et al.* Prognostic value of urinary calprotectin, NGAL and KIM-1 in chronic kidney disease. Kidney and Blood Pressure Research, 2018; 43: 1255-1262.
19. Kim SY, Jeong TD, Lee W, Chun S, Sunwoo S, *et al.* Plasma neutrophil gelatinase-associated lipocalin as a marker of tubular damage in diabetic nephropathy. Annals of Laboratory Medicine, 2018; 38: 524-529.
20. Wu J, Shao X, Lu K, Zhou J, Ren M, *et al*. Urinary RBP, and NGAL levels are associated with nephropathy in patients with type 2 diabetes. Cellular Physiology and Biochemistry, 2017; 42: 594-602.
21. Li A, Yi B, Liu Y, Wang J, Dai Q, *et al.* Urinary NGAL, and RBP are biomarkers of normoalbuminuric renal insufficiency in type 2 diabetes mellitus. Hindawi Journal of Immunology Research, 2019; 1-11.