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# Urine neutrophil gelatinase-associated lipocalin (NGAL) as an initial biomarker of acute kidney injury (AKI) in an intensive care unit (ICU) patients: a preliminary study

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## ABSTRACT

**Background:** Acute kidney injury (AKI) is one of the complications in critical patients that can increase morbidity and mortality. The condition of AKI is reversible and must be detected as early as possible. One of a potential marker for early detection is urine Neutrophil gelatinase-associated lipocalin (NGAL). This study aims to evaluate urine NGAL as an early AKI detection marker among critically ill patients.

**Methods:** This is an observational prospective cohort study involving 39 patients who were admitted to the Intensive care unit (ICU) of Dr. Sardjito General Hospital Yogyakarta during June-August 2018. The inclusion criteria were ICU patients with age>19 years old, and exclusion criteria were chronic renal failure and kidney transplantation. The AKI established based on an increase in serum creatinine levels ≥0,3 mg/dl in 48 hours or urine volume <0.5 ml /kg/hour in 6 hours. Mann Whitney test, Spearman correlation, and Chi-Square were used for statistical analysis. Data were analyzed using SPSS version 17 for windows.

**Results:** The results showed that there were no significant differences between the urine NGAL levels of AKI and non-AKI patients (130 (85-177) ng/dL; 56.65 (2.8-1500) ng/dL, p=0.407). In this study, only 3 cases of AKI were obtained from 39 subjects. Further analysis was carried out by looking at the correlation between urine NGAL levels and urine leukocyte count. The results showed a moderate correlation between them (r=0.6, p=0.001). The proportion of patients with elevated urine NGAL levels was greater in the group of patients with elevated urine leukocyte counts compared to the group with normal urine leukocyte counts (86,7%; 13,3%, p=0,008).).

**Conclusions:** Evaluation of urinary NGAL application as an early marker of AKI was not optimal in this study. An increase of urine leukocytes interfered urine NGAL.

**Keywords:** urine NGAL, early detection, AKI, intensive care unit

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## INTRODUCTION

Acute Kidney Injury was one of complication that frequently occurred in critically patient with the incidence of 20-30%. Gopulani Research in 2012 had shown that AKI incidence in a patient treated in ICU was range from 36-67%. Acute kidney injury was highly associated with high morbidity and mortality rate, prolonged hospital stay, and eventually impacting the cost of treatment.[1,2] This AKI condition was still reversible if detected in early stages. In recent protocols, diagnosis of AKI was established as creatinine level increasing and urine output decreasing that showing functional disorders. However, increasing creatinine level was not an early marker because whenever the creatinine level had been increasing, it concurrently showed kidney damage for about 50%. Therefore, creatinine level could not be used as early detection anymore. Earlier detection to an early stage of subclinical structural of the kidney will facilitate reversibility management of kidney function.[3]

Lately, it had been developed a few new markers, for example, Neutrophile Gelatinase-associated Lipocalin (NGAL).[2] NGAL was a small protein bound with neutrophil gelatinase on specific leucocyte granule that frequently expressed in the distal tubule and collecting duct. If ischemia condition occurred in tubule, NGAL level would be increasing 2-hour post ischaemia.[4] Application of Urine NGAL in Indonesia was still rarely researched.

Meanwhile, across the globe, the results were still varied. One of repercussion of those variations were tribe difference that affecting urine NGAL level among patients. Therefore, research was necessarily considered to evaluate creatinine role as early detection of AKI in a patient treated at ICU of Dr. Sardjito General Hospital. This research was aimed to assess the use of urine NGAL parameter in detecting early AKI including association and interference of urine leucocyte to urine NGAL level.

## METHODS

This research was prospective cohort research used to evaluate Neutrophile Gelatinase-associated Lipocalin (NGAL) role in the early detection of AKI. Subjects were patient treated in ICU Dr. Sardjito General Hospital with inclusion criteria either male or female, age more than 19 years old and exclusion criteria patient with Chronic Kidney Disease (CKD) or prior to undergoing kidney transplantation and patient with above normal creatinine serum level. On the other hand, AKI was defined based on increasing creatinine level ≥0,3 mg/dl in 48 hours or increasing serum creatinine level 1,5 times from baseline that had been known or predicted occurring in 7 days previously, urine volume <0,5 ml/kg/hour in 6 hours.

Urine and blood sample was drawn when the patient was treated in day 0 and day 2 in the ICU. Research subject was taken consecutively to the patient treated in ICU Dr. Sardjito General Hospital that fulfills inclusion and exclusion criteria. A blood sample was used to examine creatinine serum. Meanwhile, urine sample was used to monitor urine NGAL and routine urinalysis. Parameter NGAL was examined with Achitect analyzer device using method Chemiluminescent microparticle immunoassay.

Meanwhile, chemical and sediment analysis were used to investigate urinalysis. Urine sedimentation examination used Sysmex UF 500i device that based on flow cytometry method. Statistical analysis was using Mann-Whitney Test, Spearman Correlation test, Chi-square and Fisher's exact test. Data were analyzed using SPSS version 17 for windows.

## RESULTS

This research was involving 39 subjects that had been treated in ICU. From 39 subject, 3 of them was suffering AKI. The percentage was about 7,69%. Baseline characteristic was shown in table 1.

In this research, 18 patient was categorized as a surgical diagnoses such as post craniotomy, post laparotomy, post-SC emergency, and post-thyroidectomy. Meanwhile, 21 patient was categorized as a medical diagnoses such as hemorrhage stroke, sepsis, miliary lung tuberculosis, cancer, cerebral edema, and myasthenia gravis.

In this study, statistical analysis was done to test the difference of urine NGAL level in the first day entering ICU among patient with AKI based on an evaluation of 48 hours after entering ICU and patient non-AKI. The analysis was shown in Table 2.

The proportion of patient AKI with elevating urine NGAL level was shown in picture 1. Among 3 patient with AKI, two of them was experiencing an increasing level of NGAL urine with more that limitation of 103 ng/ml (Figure 1). Cut-off level of urine NGAL was adopted from the study of Cullen et al. in 2012 that researching urine NGAL level

**Table 1 Baseline characteristic of respondents**

| Characteristic | Total (N=39) | AKI | Non-AKI | P-Value |
|---|---|---|---|---|
| Age (Mean±SD)(years) | 50.08±21.21 | 40.67±17.15 | 51.77±21.13 | 0.35 |
| Sex (n,%) | | | | |
| Male | 20 (24.7%) | 2 (10.5%) | 17 (89.5%) | 0.362 |
| Female | 19 (23.5%) | 1 (5.3%) | 18 (94.7%) | |
| Diagnosis (n,%) | | | | |
| Surgical* | 18 (46.16%) | 2 (67%) | 16 (44%) | 1.000 |
| Medical* | 21 (53.84%) | 1 (33%) | 20 (56%) | |
| Past Medical History (n,%) | | | | |
| Hypertension* | 13 (23%) | 0 (0%) | 13 (23%) | 1.000 |
| Diabetes Mellitus* | 1 (2.5%) | 0 (0%) | 1 (2.5%) | 1.000 |
| Congestive Heart Failure* | 3 (7.6%) | 0 (0%) | 3 (7.6%) | 1.000 |
| Stroke* | 2 (5.1%) | 0% | 2 (5.1%) | 1.000 |
| Laboratory Results (Mean±SD) | | | | |
| creatinine urine day-0 | 62.6±42.1 | 61.2 (9.5-128.3) | 1567 (2.64-221.4) | 0.44 |
| creatinine serum day-0 | 0.78±0.42 | 0.68 (0.49-1,91) | 0.72 (0.42 – 1.91) | 0.46 |
| creatinine serum day-2 | 0.67(0.22-2.4) | 1.61 (1.1-2.4) | 0.62 (0.22-1.17) | 0.03 |
| eGFR (Mean±SD) | 133.46±49 | 126.9 (99.1-1) | 118.4 (35.7-163.5) | 0.47 |

*)Fisher's exact test; SD: standard deviations; eGFR: estimation glomerular filtration rate; AKI: Acute Kidney Injury; P-value: statistically significant if less than 0.05


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**Table 2** Statistical Test for Urine NGAL patient with AKI and non-AKI

| Parameter | AKI | Non-AKI | P |
|---|---|---|---|
| NGAL urin (ng/dL) | 130 (85-177) | 56,65 (2.8-1500) | 0.407* |

*Mann-Whitney Test; AKI: Acute Kidney Injury; NGAL: Neutrophil gelatinase-associated lipocalin

**Table 3** Correlation of Urine NGAL level with leucocyte urine count

| Parameter | Leucocyte urine count (cell/mm²) | |
|---|---|---|
| Urine NGAL (ng/dL) | r =0.6 | p=0.001* |

Explanation: *Spearman Correlation test

**Table 4** Patient proportion with elevating urine NGAL and urine Leucocyte

| Parameter | Leucocyte Urine Count | | P |
|---|---|---|---|
| | Normal (<25 cell/uL) | Elevating (>25 cell/uL) | |
| Normal urine NGAL Level (≤ 103 ng/mL) | 13 (54.17%) | 11 (45.33%) | 0,008 |
| Elevating urine NGAL Level (> 103 ng/mL) | 2 (13.3%) | 13 (86.7%) | |

Explanation: *: Chi Square Analysis Test

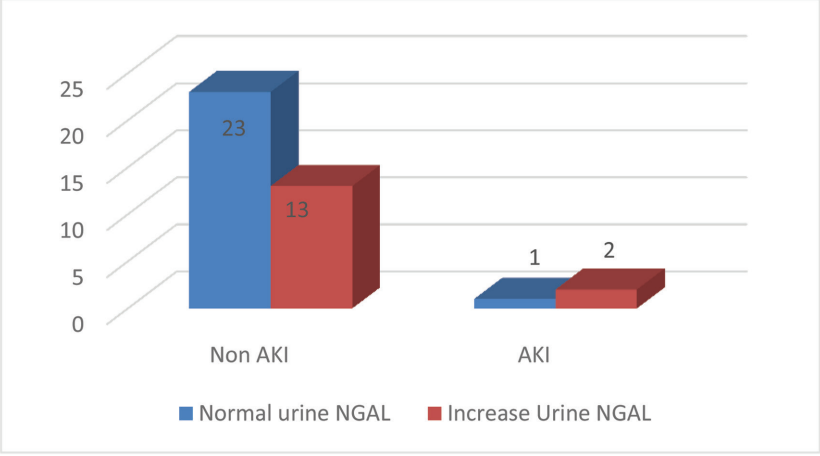


**Figure 1** The proportion of patient AKI with elevating level of urine NGAL

in a healthy population with the conclusion of 95 percentile of urine NGAL level was 103 ug/L or equal to 103 ng/mL.[5]

Among 13 non-AKI patient that has suffered elevating urine NGAL, all of them had an increasing level of urine leucocyte. To evaluate leucocyte interference thoroughly to urine NGAL, this study was also analyzing the correlation of urine NGAL and leucocyte count in urine sediment that was examined with Urine flowcytometry analyzer. The result of correlation was shown in Table 3.

This study had revealed that in non-AKI group, urine NGAL level was also elevating or equal to 103 ng/mL.[5] Statistical analysis was done to evaluate whether this urine NGAL level elevation was also obtained in a group with elevating leucocyte urine count (Table 4).

## DISCUSSION

This study had shown that the incidence of AKI in the patient treated at ICU was about 7,69%. Prevalence of AKI in this study was far lower than several previous studies, for instance, a study was done by Mahmoodpoor et al. in 2018 had shown that prevalence of AKI among patient treated at ICU of an academic hospital in Iran was about 50%. Another study done by Yegenaga et al. to the patient treated in ICU Education Hospital in Turkey was about 34%. Study of Egal et al. had obtained a prevalence rate of AKI about 35% in 72 hours of intensive care in Netherland Hospital.[6,7,8]

The difference of data in this study was affected by the time diagnosis of treatment in ICU. A study done by Egal et al. had revealed AKI in 72 hours followed by inclusion criteria oliguria 6 hours post-treated at ICU. A study done by Yegenaga, et al. had established AKI in day 2 and between day 3 and day 7 after enrolling in ICU.[6,7,8]

In this study, there was no difference between subject AKI and non-AKI based on average age. A study done by Yegenaga et al., in 2017 had shown a higher range of age, which was about 59 (18-89) y.o. That study was also revealing older patient in the AKI group compared with no AKI patient group (consecutively 62.16±17,47 and 54.48±12,27 y.o.). Other study done by Mahmoodpoor et al. (2018) had shown no difference in age between AKI and non-AKI group (consecutively 65.1±12.27 and 60.4±8.6 years old).[6,7]

In this study, it was revealed that there was no difference in gender proportion between the patient with AKI and non-AKI. This founding was similar with other study done by Yegenaga et al. (2017) that had shown there was no difference of male and female proportion between AKI and non-AKI group (consecutively 55.60%; 52.5% for male and 44.4%; 47.5% for female, p=0.694).[6]

This study had shown that there was no significant difference in creatinine serum level and urine creatinine in day 0 of a patient with AKI and non-AKI. Nevertheless, there was a difference found in serum creatinine level day 2 inpatient AKI and non-AKI, which is consecutively 1.61 (1.1-2.41); 0.62 (0.22-1.17), p=0.003. Another research done by Yegenaga et al. (2017) had shown that creatinine serum level was higher in early day enrolling to ICU between a patient with AKI and no AKI (Consecutively 1.98±1.2 mg/dL dan 0.72±0.2 mg/dL). Meanwhile,

a study done by Mahmoodpoor et al. (2018) had shown that there was no difference between serum creatinine level day 0 between a patient with AKI and non-AKI, consecutively 2.7 (IQR: 0.7); 2.7 (IQR: 0.8), p=0.895.[6,7]

This study was also analyzing urine NGAL level in the patient when enrolled to the ICU for the first time. This study had revealed that there was no difference significantly between patient AKI and non-AKI. This study was resembled Mahmoodpoor et al. that had been showing no difference between urine NGAL level in a patient with AKI and non-AKI consecutively 65 (IQR:20) and 61 (IQR:20) ng/mL. Other research was also showing that urine NGAL level in a patient with AKI was statistically higher than non-AKI (consecutively is 59.72 – 24.82-69.1 ng/mL, p=0.001).[6,7]

Urine Neutrophile Gelatinase-associated Lipocalin (NGAL) was a small protein bound to neutrophile gelatinase specific leucocyte granule. NGAL was also expressed by epithelial tissue as a part of anti-microbe mechanism. In normal kidney, NGAL was only expressed in the distal tubule and collecting duct. In AKI condition, kidney proximal tubule cell was also expressing NGAL that derived from NGAL reuptake that was enormous in glomerulus filtrate. NGAL was expressed at a low level in some tissue such as lungs, large intestine, and epithelial cell located in the proximal tubule.[9]

A study was done by Mahmoodpoor et all failed to prove urine NGAL capability in predicting AKI. Meanwhile, plasma NGAL that was measured when enrolling to ICU was able to predict AKI incidence by the cutoff 113 ng/ml with an area under the curve (AUC) 0,723±0,073, p=0,006. In that study, plasma NGAL level in the patient was different between a patient with AKI and not AKI (consecutively 129 (IQR: 20); 111 (IQR: 32). Urine NGAL level was undergoing decreasing in day 3 until day 7.[6]

One of factor interfering that results was leucocyte urine. This study had proven a medium correlation between NGAL urine level and leucocyte urine count. Patient with the elevating level of NGAL urine was obtained more highly in a subject with elevating leucocyte count. This result was similar with the study done by Koo et all that researched efficacy urine NGAL in predicting the severity of AKI post nephrectomy. Results of Research Koo et al. had shown there was no correlation between NGAL urine with AKI severity, although urine NGAL tended to similar with NGAL plasma. One of the causes suspected was the process of nephrectomy would cause naturally induce inflammation and elevate urine leucocyte level that had an impact in measuring NGAL.[10] NGAL was one of acute protein phase that first time identified on neutrophile granule and could be excreted by activated neutrophils.[11] This condition would cause elevating urine NGAL in the condition of elevating urine leucocyte. Other study associated with urine leucocyte influence particularly neutrophile to urine NGAL level, was done by Proverbial et al. had shown that median NGAL level was higher in patient urine leucocyte count >5/HPF, higher compared with group with leucocyte count <5/HPF consecutively 23.65 pg/mL (20.04–29.80) and 4.96 pg/mL (0.29–11.34), p=0.0053.[12] Another study done Yilmaz et al. in 2009 researching urine NGAL level in children patient with Urinary tract infection (UTI) had shown that urine NGAL level of patient with UTI was higher than patient without UTI (91.02 ng/mL vs. 14.29 ng/mL, p=0.0001). This condition was related to neutrophile activity in UTI that increased urine NGAL level.[13]

## CONCLUSION

In this study, evaluation of urine NGAL in detecting AKI had not been yet showing optimum result and obtained a correlation between urine NGAL level and sediment urine. The study should be conducted with more extended monitoring and evaluation. In order to observe urine leucocyte interference, urine NGAL observation was better being followed by urinalysis examination to determine intervention of urine leucocyte.

## ETHICAL CLEARANCE

This research had been approved by the Ethics Committee prior to the study conducted.

## CONFLICT OF INTEREST STATEMENT

The authors declare that there was no conflict of interest in this research.

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## AUTHOR CONTRIBUTION

All authors have contributed to all process in this research, preparation, drafting, review, and approval of this manuscript.

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