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Neutrophil Gelatinase-Associated Lipocalin (NGAL) in Urine as a Potential Non-Invasive Method for Determine Kidney Damage in Predialysis Patients

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KEYWORDS

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Abstract NGAL expression in acute kidney failure is well known. It is approved that NGAL expression occurs earlier than the level of BUN and creatinine in acute kidney failure. NGAL is not only expressed in the blood but also in the urine, where urine collection has many advantages over blood collection. This study aims to observed the expression of NGAL in predialysis patients and to determine the correlation between NGAL serum and NGAL urine of patients. The sample was taken from healthy persons as control and predialysis patients. The examination of BUN, creatinine, and urinalysis were done to approve the diagnose in predialysis patients. While NGAL in serum and urine were analyzed using the Enzyme-Linked Immunoabsorbent Assay (ELISA). The results showed that the concentration of NGAL in serum was higher in the predialysis patients compared to the healthy subjects ($p<0.05$). There was a strong positive correlation between the NGAL in the serum and the NGAL in the urine ($r= 0.98$ and $p<0.000$). It is concluded that the non-invasive examination of NGAL in urine can be choose rather than using serum NGAL. However, it must be noted that NGAL could be used for chronic renal failure in predialysis patients as long as other biomarkers have been proven.

Introduction

Predialysis patients are patients who will undergo hemodialysis due to the inability of the kidneys to remove metabolic waste. One of the gold standards for hemodialysis is to monitor the increase in BUN and creatinine levels. But the expression of these two biomarkers tends to appear after kidney function has decreased progressively. This has led many researchers to look for biomarkers that are earlier expressed after kidney injury such as NGAL which appears shortly after injury and before serum creatinine elevation (Endre *et al*., 2008). NGAL can accumulate in the blood and urine (Mori *et al*., 2005), while urine examination is the best choice because it is non-invasive method. Recent clinical studies show that NGAL urine reflects acute renal damage early (Devarajan, 2006, Mishra *et al*., 2003) but may also signal chronic renal damage (Mishra *et al*., 2005; Wagener *et al*., 2006) as suffered by predialysis patients (Ratnawati *et al*., 2017). But whether the amount of NGAL in the urine can describe the number of NGAL in the blood has not been agreed. This study aims to evaluate the relationship of NGAL expression in urine and serum samples in predialysis patients.

Materials and Methods

Predialysis Patient

Urine and blood samples were collected from 30 adult patients predialysis from the internal medicine policlinic of Dr. Saiful Anwar Hospital Malang Indonesia. Additionally, another 30 urine samples were collected from

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healthy people and served as normal controls. Patients predialysis were taken the sample prior to renal dialysis procedures began.

Assay of Blood urea nitrogen and Creatinine

Serum levels of blood urea nitrogen and creatinine were measured by spectrophotometer in the Clinical pathology laboratory of Medical Faculty, University of Brawijaya (blood chemistry, Horiba).

Enzym Linked Immunosorbent Assay (ELISA)

The urine was immediately centrifuged at 3000 rpm for 15 min at 4 °C. Serum was obtained by centrifugation of the blood at 3000 rpm for 15 min at 4 °C. All sample supernatants were immediately stored in aliquots at −20 °C. Serum and NGAL urine was analyzed with a commercial sandwich ELISA kit, (BioLegend, San Diego Cat. No. 562401). ELISA Human NGAL was carried out according to the manufacturer's protocol. The readout was performed using a microplate reader (Bio-Rad Instruments, USA).

Data analysis

Level of urea and creatinine were analyzed by SPSS 17 statistical program, data normality test using Kolmogorov-Smirnov test and data were analyzed by independent T test in group healthy samples vs predialysis patient (BUN, creatinine and NGAL serum) and Pearson correlation test for predialysis patient (NGAL serum vs NGAL urine, NGAL serum vs BUN and NGAL serum vs creatinine).

Results and Discussion

BUN and creatinine serum in healthy and predialysis samples

Until now BUN and creatinine are still commonly used as kidney damage biomarker but it will appear when kidney damage has reached 80%. BUN and creatinine in healthy samples in this study were 10-33 mg/dL and 0.2-1.4 mg/dL respectively. While in predialysis patients was 55-278 mg/dL (BUN) and 1.8-9.9 mg/dL (creatinine). Statistical results showed an increase in bun and creatinine in predialysis patients higher than in the healthy human group ($p < 0.01$). BUN and creatinine increase in the predialysis patient is caused by renal function decrease as a place for filtration and excretion product metabolism from the body.

Comparison of NGAL serum between healthy and predialysis samples

NGAL concentration from healthy people in this study was 17.21 ng/mL ± 0.37; whereas the predialysis patient was 18.28ng/mL ± 0.14. The independent T-test both groups differed significant ($p<0.05$) (Figure 1). In a healthy person showed the presence of NGAL serum, this was due to NGAL was synthesized during granulocyte maturation in the bone marrow and increased expression occurs in epithelial cells inflamed or malignancy. In humans, NGAL normally exposed at very low levels in some tissues such as kidney, lung, stomach, and colon (Borregaard & Cowland, 2006; Xu & Venge, 2000). While NGAL concentration in predialysis patients showed increase up to 1.0 ng/mL compared to healthy group. In the incidence of kidney disease NGAL expression was increased, renal epithelial tissue damage in turn would activate NGAL gene which was known an upregulated genes in renal failure condition of ischemia in mice (Schmidt-Ott, 2011). NGAL serum in this study was very low compared with NGAL serum after abdominal aortic aneurysm (78–200) ng/mL) (Ramos-Mozo, 2011), patient undergo heart and lung transplant (168.5 ± 73.1 ng/mL) (Szewczyk *et al*., 2009), and in AKI patient with septic shock (216 ng/mL), acute decompensated heart failure (97-292 ng/mL) (Aghel *et al.*, 2010).

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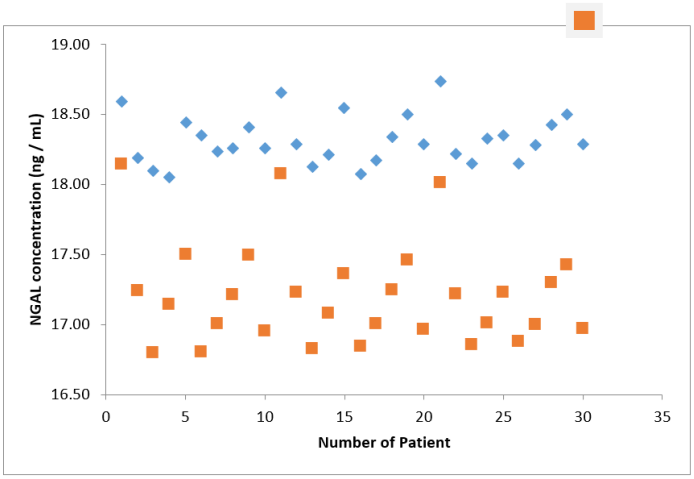

Figure 1. NGAL serum level in predialysis showed higher result (■) and healthy person (◆) (p<0,01)

Relationship between BUN and Creatinine, NGAL serum, NGAL urine, in predialysis samples

BUN and creatinine in predialysis patient was elevated compared with normal patient samples. The increase in BUN value would be followed by an elevating of creatinine since it was associated with the retention of both compounds due to kidney damage. However, there was a negative correlation with NGAL serum and BUN serum (r=0.386, p=0.345) neither NGAL serum and creatinine serum (r=0.253, p=0.544). Despite NGAL concentration in this study was also increased in the predialysis patient. Our finding was similar with Helmersson-Karlqvist *et al.* (2013). Neutrophil gelatinase-associated lipocalin in the urine may be a biomarker for early prediction of acute renal damage (Pedersen *et al*., 2010). NGAL proteins are easily detectable in serum and urine in animal models of mice (Mishra *et al.,* 2004; Han et al. 2012). A study conducted by Devarajan *et al.* (2003) and Nickolas *et al.* (2008) indicated that detection of the presence of NGAL protein in serum and urine was a sensitive and specific biomarker in predicting the incidence of acute renal failure. However, the ideal biomarker for acute renal damage should be noninvasive, such as using a urine sample as an initial diagnosis of impaired renal function our study using independent T-test results showed that NGAL serum levels were not differenced with NGAL urine concentrations (p>0.05). The relationship between NGAL serum and NGAL urine was significant (r=0.98 dan p<0.000). It meant that NGAL in serum was expressed similarly in the urine. These results support the study of meta-analysis in eight countries by Haase *et al.* (2009) that the accuracy of NGAL on serum or blood serum is similar to that of the urine. NGAL urine as an early marker kidney disease was reported by Mishra *et al.* (2005) in urine children undergo cardiac surgery and also in multiple trauma patients even in first-day injury (Makris *et al*., 2009). However, we do also understand that NGAL in blood serum expresses overall body NGAL production (systemic NGAL balance) not only renal NGAL production, therefore we must confirm that predialysis patients did not undergo a pathological condition than will disseminated renal NGAL expression.

Conclusions and Suggestion

It can be concluded that the expression of NGAL biomarkers in urine showed similar results with NGAL expression in blood serum. Our finding suggested that NGAL protein in the urine can be used as a non-invasive biomarker of kidney disease in predialysis patient.

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