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# Effect of Intravenous Vitamin C on Urine Neutrophil Gelatinase-Associated Lipocalin Among Septic Patients: Randomized Controlled Trial

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**Abstract**

**Background**
The role of vitamin C to decrease organ dysfunction in sepsis was still controversial. This study aimed to explore the effect of intravenous (IV) vitamin C on urine NGAL (uNGAL) levels among septic patients in ICU.
**Methods**
This study was a randomized clinical trial held in Cipto Mangunkusumo Hospital from April to July 2019 with consecutive sampling method. Patients aged > 18 years with sepsis based on the criteria of sepsis-3 who were admitted to the ICU were included in this study. Exclusion criteria were those with chronic kidney problems, with kidney stones, undergo renal replacement therapy in the ICU. All subjects were divided into: Group A was treated with combination of vitamin C and thiamine while Group B was given thiamine only. The uNGAL level was measured at baseline, 24, 48 and 72 h after treatment. Anova for repeated measurement using General Linear Model for Repeated Measurement was used with level of significant at p-value <0.05.
**Results**
Total of 33 subjects were included. In Group A we found uNGAL (ng/ mL) were 74.5 (13.3-102.9), 77.3 (15.2-98.4), 67.2 (22.6-100.6), 77.2 (17.0-100.5) for baseline, 24 h, 48 h, and 72 h respectively. While in Group B uNGAL were 57.7 (11.5-94.5), 57.1 (6.4-97.7), 53.7 (13.3-99.6), 47.6 (4.5-100.9). No significant difference in terms of uNGAL between two groups at each hour was found.
**Conclusions**
This study showed that intravenous vitamin C administration had no effect on uNGAL among septic patients. Need more study to investigate approaches to improve kidney and inflammatory biomarker among septic patients.

**Keywords:** *sepsis, intravenous vitamin C, urine NGAL*

## Introduction

Sepsis is caused by dysregulated host response to infection which leads to life-threatening organ dysfunction.[1] Mortality due to sepsis in critically ill patients is around 30-50%.[2] Fifty-one percent of sepsis patients develop acute kidney injury (AKI), which is very

serious complication in intensive care unit (ICU) that morbidity, mortality and treatment costs would increase.[3] Currently, diagnostic criteria of AKI was based on Acute Kidney Injury Network (AKIN) classification.

Oxidation contributes to increased highly reactive species free radicals production. This would cause further chain reactions which leads to cell damage, including apoptosis in kidney tubule cells.[4,5] It is possible that antioxidant supplementation may provide potential advantages due to the role of oxidative stress in the pathogenesis of sepsis. The most studied antioxidant molecule is vitamin C. Studies showed intravenous vitamin C was effective at reducing mortality and organ injury by using pleiotropic mechanisms. High-dose ascorbate administration can counteract the oxidative phosphorylation uncoupling leading to reactive oxygen species (ROS) reduction.[4,6]

Neutrophil gelatinase-associated lipocalin (NGAL) is the most widely published biomarker. Elevated urine NGAL is a signal of early kidney function decline which is reflected by increased creatinine.[7]

However, current results of past studies involving antioxidant supplementation, including vitamin C, were highly varied. Study by Heyland et al showed administration of vitamin C (1500 mg/ day) combined with other antioxidants failed to improve survival in critically ill adults. Contradictory, Marik et al showed extreme mortality reduction in septic patients receiving the vitamin “cocktail” and reduction of renal replacement therapy event between treatment group and placebo (10% vs 33 %, p=0,02).[8,9] Hence, we would evaluate role of vitamin C in critical patients by using randomized clinical trial. This study aimed to explore the effect of intravenous vitamin C on uNGAL levels among septic patients in ICU.

## Methods

This study was a randomized clinical trial held in Cipto Mangunkusumo Hospital from April to July 2019 with consecutive sampling method. Patients aged > 18 years with sepsis diagnosis based on sepsis-3 criteria who were admitted to the ICU and who were in between 6 h and 24 h post resuscitation after sepsis diagnosis in ICU were included in this study. Meanwhile, exclusion criteria were patients with chronic kidney problems on hemodialysis, , kidney stones or kidney problem within the last 3 months. The drop out criteria were patients

underwent renal replacement therapy in the ICU within 72 h observation and who were given corticosteroid within 72 h. A written consent was obtained from all study participants.

Subjects were determinated by simple randomization using random number table and divided into two groups. Patients in Group A was administered IV vitamin C 1,5 grams per 6 hours and thiamine 200 mg per 12 hours. In Group B was given thiamine only. The treatment was given on first day until 3rd day in ICU. Baseline demographic data was recorded on the first day. Sequential Organ Failure Assessment (SOFA) score was recorded at baseline, 24, 48 and 72 h after treatment. Daily measurements of uNGAL was started as baseline level and continued at 24, 48 and 72 h after treatment. The enzyme-linked immunosorbent assay kits were utilized for measuring urine NGAL (Quantikine® ELISA R&D systems, Minneapolis) and the level was defined as their total consentrations in urine (ng/mL). The outcome of study was the change in uNGAL levels over the 72-hours. SPSS software version 23.0 (SPSS Inc, Chicago, III, USA) was used to perform statistical analysis. For continuous variables, results were displayed as mean values ±standard deviations for normally distributed data and median (minimum-maximum) for not normally distributed data. For categorical data, result used frequency and percentages. Comparison between both groups was analysed by using Mann Whitney test for numerical data and Fisher test for categorical data. Anova for repeated measurement using General Linear Model for Repeated Measurement was used for serial measurement analysis. Wilcoxon pair comparison test was done for analyzing before and after treatment effect. Level of significant was determined at p-value <0.05.

## Results

**Table 1. Baseline demographic data of Group A and B**

| | | Group A (n=18) | Group B (n=15) |
|---|---|---|---|
| Age, mean±SD,y | | 45.39±15.06 | 46±16.6 |
| Sex, male, (%) | | 10(55.6) | 7(46.7) |
| Diagnosis, (%) | Sepsis | 7(38.9) | 6(40) |
| | Septic shock | 11(61.1) | 9(60) |
| Comorbidities, (%) | No | 7(38.9) | 4(26.7) |

| | | | |
|---|---|---|---|
| Source of sepsis, (%) | Abdominal | 3(16.7) | 4(26.7) |
| | Pulmonary | 14(77.8) | 10(66.7) |
| | Soft tissue | 1(5.6) | 1(6.7) |
| | Medical | 9(50) | 10(66.7) |
| | Surgical | 4(22.2) | 4(26.7) |
| | Medical and surgical | 5(27.8) | 1(6.7) |
| Type of surgery, (%) | Abdominal | 5(27.8) | 4(26.7) |
| | Neurosurgery | 2 (11.1) | 0 (0) |
| | other | 2 (11.1) | 1(6.7) |
| Mechanical ventilation (yes), (%) | | 18 (100) | 14(93.3) |
| P/F ratio, (%) | >300 | 10(55.6) | 5(33.3) |
| | 200-300 | 5(27.8) | 6(40) |
| | <200 | 3(16.7) | 3(20) |
| Vasopressor (no), (%) | | 6(33) | 3(20) |
| AKI (yes)*, (%) | | 0 | 0 |
| SOFA score baseline, mean±SD | | 7.56±2.87 | 9.07±2.34 |
| uNGAL baseline, mean±SD, ng/mL | | 66.06±30.25 | 55.78±31.12 |

* Based on urine output criteria by AKIN classification

Forty patients were included in this study, 7 patients were dropped out because they passed away before 72 h observation. Baseline demographic data of two groups were presented in table 1. This study did not find any significant differences between two groups regarding these characteristics.

There were no significant differences in uNGAL between the two groups at each hour respectively (table 2). There were also no significant changes in uNGAL level in before and after treatment analysis either in Group A (p=0.811) or in Group B (p=0.865) (figure 1).

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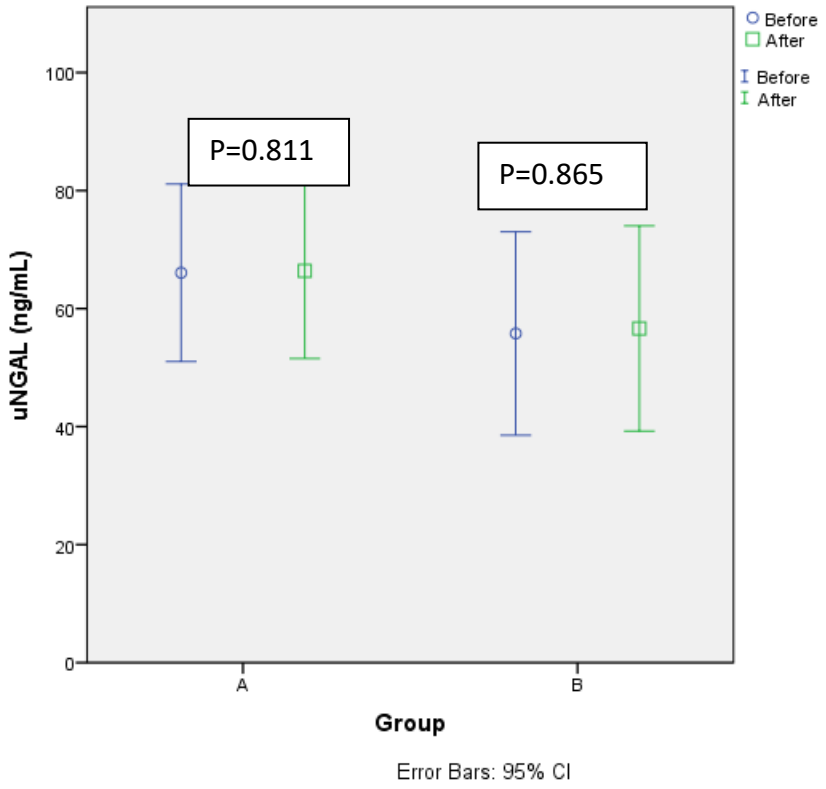


**Figure 1 : Changes in uNGAL at before and after treatment in Group A and Group B**

**Table 2. uNGAL levels in the two study groups**

| Characteristic | Group A Median (IQR) | Group B Median (IQR) | p-value |
|---|---|---|---|
| uNGAL (ng/mL) | | | |
| Baseline | 74.5(13.3-102.9) | 57.7(11.5-94.5) | 0.345 |
| 24-hours | 77.3(15.2-98.4) | 57.1(6.4-97.7) | 0.283 |
| 48-hours | 67.2(22.6-100.6) | 53.7(13.3-99.6) | 0.730 |
| 72-hours | 77.2(17.0-100.5) | 47.6(4.5-100.9) | 0.368 |

**Discussion**

Based on AKIN classification, all subjects in this study were not in AKI condition at ICU admission. However, this would be different if using NGAL parameter. A research evaluating plasma NGAL for early AKI diagnosis in a heterogenous adult patients concluded nearly 30% had AKI within the first day of admission. Additionally, there were 44% of patients had AKI during their ICU admission.[3,10] From uNGAL data, there was very wide range of levels was found in general ICU population. Endre et al found similar results in their study.[11] uNGAL could be measured in critically ill patients within the first day of admission. However, this may not indicate the first day from the onset of the disease itself. In the ICU, "multiple injuries" of varying severity might impact the kidney in the process; this was different from the "single injury" model found in most patients underwent surgery.[12] Additionally, NGAL is also served as a marker of bacterial infection and systemic inflammation which was released by neutrophil activation.[7] However, systemic stress could increase uNGAL excretion in the absence of AKI which generated substantial extrarenal NGAL; similar findings may also found from both chronic and acute renal illness.[13] Hence, for future reference, combinations of biomarkers, including liver fatty acid-binding protein (L-FABP) and cystatin-C, could allow AKI diagnosis among septic patients.[11]

This study is currently the first trial that explores the effect of IV vitamin C on uNGAL in septic patients. However, there was no significant changes in uNGAL level in before and after treatment analysis in two groups. The reason might be high-dose intravenous vitamin C should be limited to only early phase of sepsis conditions. This is because intracellular signalling is initiated by low level of ROS.[14] However, in this study majority of subjects came from tertiary referral hospital with complex medical problems; hence, the subjects were already in long-term release of ROS. Further studies should exclude patients with terminal end stage disease, including those who are less likely to survive, patients with sepsis condition more than 24 hours before admission or those who were already treated in or referred from other institutition.

Abiles et al studied the association between oxidative stress, vitamin intakes, and SOFA scores among critically ill patients. This study found worsened oxidative stress among patients with antioxidant vitamin intakes below recommended dietary allowance (RDA). Hence, dose adjustment should be personalised based on the level of each patient's antioxidant level. Additionally, antioxidant vitamins intakes should be monitored carefully to personalise the

dose in accordance with the RDA.[15] However, there were variable responses toward vitamin C administration among critically ill patients.[16] For future reference, there are several issues remain unsolved including pharmacokinetics of vitamin C and other antioxidants supplementation.

## Conclusion

This study showed that intravenous vitamin C administration had no effect on uNGAL among septic patients. Further study was required to investigate the role of vitamin as antioxidant in critically ill patients.

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