

Urinary Neutrophil Gelatinase-Associated Lipocalin in Type 1 and Type 2 Hepatorenal Syndrome and Its Correlation with Kidney Function

Lider Olmen Panggabean¹, Gontar Alamsyah Siregar², Dharma Lindarto³,
Masrul Lubis²

¹Department of Internal Medicine, Faculty of Medicine, Sumatera Utara University, Medan

²Division of Gastroenterohepatology, Department of Internal Medicine, Faculty of Medicine, Sumatera Utara University, Medan

³Division of Metabolic and Endocrinology, Department of Internal Medicine, Faculty of Medicine, Sumatera Utara University, Medan

Corresponding Author: Gontar Alamsyah Siregar

ABSTRACT

Objective: To determine the difference in urinary Neutrophil Gelatinase-Associated Lipocalin (uNGAL) levels in type 1 and type 2 Hepatorenal Syndrome (HRS) and determine the correlation between urinary NGAL and kidney function and the degree of liver disease severity in HRS patients.

Methods: A total of 26 patients consisting of 13 patients in each group, both in type 1 and type 2 HRS at Adam Malik Hospital, Medan City, Indonesia. Urine samples were taken for NGAL examination using the ELISA method. A blood sample is used to assess kidney function. Liver disease severity was measured using the Child-pugh score. Data were analyzed using statistical t-test independent and Pearson correlation test. A *p* value less than 0.05 was applied to each statistical test as significant.

Result: Total subjects consisted of 18 men and 8 women, with a mean age 57.5±8.91 years old. Hepatitis B virus was the most common etiology of liver cirrhosis in this study (53.8%) and the most degree of liver disease severity was Child-pugh C (65.4%). Urinary NGAL levels in type 1 HRS is significantly higher than type 2 (155.71±43.04 vs 105.85±32.32 ng/ml, *p* = 0.003). There was a positive correlation between uNGAL and serum creatinine (*r* = 0.488, *P* = 0.006). There was a positive correlation between uNGAL and Child-pugh score (*r* = 0.466, *P* = 0.008).

Conclusion: Urinary NGAL level in type 1 HRS is higher than type 2, there is a positive correlation between uNGAL and serum creatinine and Child-pugh score in HRS patients.

Keywords: Hepatorenal syndrome, urinary NGAL, Liver cirrhosis, kidney function.

INTRODUCTION

HRS is a complication that often occurs in Liver cirrhosis. Gines et al. reported the incidence of HRS in liver cirrhosis patients reaching 18% in the first year and will increase to 39% by the fifth year. [1] A study conducted by Salerno *et al.* on liver cirrhosis patients with ascites at 21 hospitals in Italy found that the prevalence of HRS was around between 13 - 45.8%. [2] A study conducted by Martin-Llahi *et al* in patients with Liver cirrhosis found that the prevalence of HRS was 13%. [3]

HRS is a specific form of acute kidney injury (AKI) that occurs in cirrhosis. Serum creatinine has limitations in distinguishing the types of AKI that occur in cirrhosis. One of the points on the International Ascites Club (IAC) criteria for the diagnosis of HRS is that it takes 48 hours to assess whether there is improvement in serum creatinine or not after diuretic administration and volume

expansion with albumin. This can delay therapy of the HRS itself and exacerbate portal hypertension.^[4,5]

Due to the limitation of serum creatinine in diagnosing HRS, a new biomarker is needed that can quickly differentiate HRS from other AKI in liver cirrhosis patients, one of which is uNGAL. NGAL is a protein with 25 kD molecular weight bound to gelatinase from neutrophil cells.^[6] NGAL is produced in the distal nephron (thick ascending limb of Henle's loop, distal tubule, collecting tubule) and its production increases in response to renal injury.^[7,8] The increase in NGAL concentration was faster than the increase in serum creatinine in AKI conditions. NGAL is also more sensitive to the presence of tissue damage than functional disorders so that it can distinguish structural or functional disorders.^[9]

Previous studies have reported that uNGAL can be useful in differentiating between HRS and other AKI that occur in HRS, this is because the level is significantly different from other AKI.^[10-14]

Meanwhile, studies comparing uNGAL levels between type 1 and type 2 HRS are still rare. There are still differences in several previous studies regarding the uNGAL level for each type of HRS. Research conducted by El-Bassat *et al.* concluded that there was a significant difference in uNGAL values between type 1 and type 2 HRS.^[13] Meanwhile, other studies reported no significant difference between uNGAL levels for the two types of HRS.¹⁰ For this reason, this study was conducted to see how the difference in uNGAL levels between types 1 and type 2 HRS and to see the correlation between uNGAL and kidney function and the degree of liver disease severity in liver cirrhosis patients with HRS.

METHODS

Study design

This study was a cross-sectional study conducted at Adam Malik Hospital, Medan city, Indonesia and Network

Hospital after obtaining approval from the University of North Sumatera Medical Research Ethics Commission / Adam Malik Hospital. The research time was carried out from May to December 2020.

Study subject

The research subjects were liver cirrhosis patients with HRS who came to the Adam Malik Hospital and Network Hospital between May - December 2020. Then these patients were grouped into the type 1 or type 2 HRS group until the number of samples was met. The diagnosis of HRS was made using the revised International Ascites Club (IAC) 2007 criteria. Patients <18 years old, pregnant women, suffering from systemic disease, sepsis, malignancy, previous history of kidney disease, and refusal to participate were excluded from this study.

Based on the IAC criteria, HRS is enforced if there is a condition, namely liver cirrhosis with ascites, Serum creatinine >1.5 mg/dl (133 µmol/L), no improvement in serum creatinine (decrease to level of <1.5 mg/dl) at least 2 days after diuretic administration and volume expansion with albumin, no shock and use nephrotoxic drugs, no renal parenchymal disease characterized by proteinuria >500 mg/day, microhematuria >50 red blood cells/high power field, and/or abnormal ultrasound. Type 1 HRS is HRS with progressive decline in renal function, with serum creatinine > 2.5 mg/dl at the time of examination and/or a 2-fold increase over 2 weeks. Type 2 HRS is HRS with a moderate and stable decline in renal function, with serum creatinine between 1.5 to 2.5 mg / dl).^[4]

Kidney function was measured by examining serum ureum and creatinine and calculating glomerular filtration rate (GFR) using formula Cockcroft and Gault. Liver disease severity was assessed using the Child-pugh score.

Biochemical Analysis

Patients who were included in the type 1 and type 2 HRS groups were

subjected to a urine NGAL examination. The middle portion of urine (spot urine) is collected at least 30 cc, then measurements are taken using commercial NGAL (Quantikine ELISA; Human Lipocalin-2 / NGAL Immunoassay, R&D System Inc). Demographic and biochemical data including complete blood count, kidney function, liver function, hematologic function, albumin, electrolytes were collected when the patient was admitted to the hospital. The Child-pugh score was calculated using clinical and laboratory data when the patient was admitted.

Statistical analysis

Data is presented descriptively, by displaying the frequency distribution and percentage for categorical data. Meanwhile, numerical data is presented by displaying data mean and standard deviation. The data normality test used the Shapiro Wilk test. To determine the difference in uNGAL levels between type 1 and type 2 HRS, an independent t-test was used, while the correlation between uNGAL with renal function and child-pugh score were assessed using the Pearson correlation test. Data analysis used the SPSS 22 (Statistical Product and for Social Sciences) computer program and a 95% confidence interval, where $p < 0.05$ was statistically significant.

RESULTS

Table 1. Characteristics of patients with HRS

Characteristics	n = 26
Gender, n (%)	
Men	18 (69.2%)
Women	8 (30.8%)
Age (years), mean ± SD	57.5 ± 8.91
Body weight (kg), mean ± SD	59.04 ± 5.99
Etiology of cirrhosis, n (%)	
Hepatitis B	14 (53.8%)
Hepatitis C	1 (3.9%)
Others	11 (42.3%)
Child-pugh classification, n (%)	
A	-
B	9 (34.6%)
C	17 (65.4%)

The total study subjects were 26 patients consisting of 13 patients in each of the type 1 and type 2 HRS groups. Most of the sexes were male, amounting to 18 patients (69.2%), with an average age of

57.5 years old. The most common etiology of liver cirrhosis was hepatitis B virus as many as 14 patients (53.8%) with the highest degree of liver severity being child-pugh C as many as 17 patients (65.4%) as shown in Table 1.

Based on the type of HRS, the most data were male, both in type 1 and type 2 HRS. The most common etiology of cirrhosis in type 1 HRS was other causes (other than hepatitis B and C) by 8 patients (61.5%) and in type 2 HRS, the most common cause of cirrhosis was hepatitis B with 10 patients (76.9%). The highest degree of liver disease severity in both type 1 and type 2 HRS was child-pugh C as shown in table 2.

Table 2. Characteristics of samples by type of HRS

Characteristics	HRS	
	Type 1 HRS (n = 13)	Type 2 HRS (n = 13)
Gender, n (%)		
Men	9 (69.2%)	9 (69.2%)
Women	4 (30.8%)	4 (30.8%)
Age (years), mean ± SD	57.54 ± 7.18	57.46 ± 10.68
Body weight (kg), mean ± SD	56.31 ± 5.46	61.77 ± 5.35
Etiology of cirrhosis, n (%)		
Hepatitis B	4 (30.8)	10 (76.9)
Hepatitis C	1 (7.7)	-
Others	8 (61.5)	3 (23.1)
Child-pugh classification, n (%)		
A	-	-
B	4 (30.8)	5 (38.5)
C	9 (69.2)	8 (61.5)
Score	10.38 ± 1.38	9.85 ± 1.34

Table 3. Characteristics of laboratory results

Laboratory Characteristics	HRS		
	Type 1 HRS (n = 13)	Type 2 HRS (n = 13)	P value
Hb	8.63 ± 1.13	9.45 ± 1.17	0.085 ^a
Leukocytes	6550 (3620 - 12340)	9470 (4940 - 12220)	0.166 ^b
Platelets	130769 ± 48499	140384 ± 48977	0.620 ^a
Na	129.77 ± 9.23	131.85 ± 7.15	0.527 ^a
K	3.9 (3,1 - 7)	4.7 (3,4 - 6,9)	0.090 ^b
Cl	102 (75 - 107)	101 (81 - 106)	0.502 ^b
Ureum	93.01 ± 27.28	75.55 ± 38.73	0.196 ^a
Creatinine	2.99 ± 0.28	1.91 ± 0.26	0.001 ^a
GFR	20.69 ± 3.56	36.38 ± 8.70	0.001 ^a
Albumin	2.52 ± 0.62	2.80 ± 0.65	0.268 ^a
Total bilirubin	3.1 (0.9 - 25.5)	3.1 (0.5 - 13.4)	0.663 ^b
SGOT	87 (23 - 328)	76 (13 - 325)	0.538 ^b
SGPT	54 (22 - 128)	49 (18 - 196)	1 ^b
INR	1.62 ± 0.48	1.46 ± 0.39	0.363 ^a

^a normal distribution data, mean ± SD
^b data distribution is not normal, median (min-max)

Table 3 shows the laboratory results of both type 1 and type 2 HRS groups. There were no significant differences from the laboratory parameters of the two groups

except for renal function parameters, where there was a significant difference in serum creatinine levels between type 1 and type 2 HRS (2.99 ± 0.28 vs 1.91 ± 0.26 ; $p < 0.001$) and also the GFR values (20.69 ± 3.56 vs 36.38 ± 8.70 ; $p < 0.001$).

There is a significant difference in uNGAL levels between type 1 and type 2 HRS, where the average uNGAL level on type 1 HRS was 155.71 ± 43.04 ng/ml and the average uNGAL level on type 2 HRS was 105.85 ± 32.32 ng/ml, with p value = 0.003 as shown in table 4.

Table 4. Urinary NGAL levels for type 1 and type 2 HRS

HRS	Urinary NGAL (ng/ml)	P value
Type 1 HRS	155.71 ± 43.04	0.003 *
Type 2 HRS	105.85 ± 32.32	

* $p < 0.05$ (significant)

Table 5 shows that there is a positive correlation between the uNGAL and serum creatinine ($r = 0.488$, $P = 0.006$). Meanwhile, there was a negative correlation between the uNGAL and GFR ($r = -0.338$, $P = 0.046$). Meanwhile, there was no correlation between urea and uNGAL ($r = 0.235$, $P = 0.124$). In terms of liver disease severity, there was a positive correlation between uNGAL and the Child-Pugh score ($r = 0.466$; $P = 0.008$).

Table 5. Relationship between uNGAL levels and kidney function and liver disease severity

	Urinary NGAL levels	
	r	P value
Serum creatinine	0.488	0.006 *
Ureum	0.228	0.131
GFR	-0.338	0.046 *
Child-pugh score	0.466	0.008 *

* Pearson correlation

DISCUSSION

Previous studies have reported that urinary NGAL can differentiate between HRS and other AKIs that occur in cirrhosis. This is because in HRS there is an increase in urinary NGAL level in the intermediate range, which is significantly different from pre-renal azotemia and acute tubular necrosis (ATN).^[10-14] The mechanism of increased NGAL in HRS patients is still unclear, it was previously known that HRS is a functional renal impairment, not a structural one.^[15] However pathological investigations have reported minor tubular

and glomerular damage in the kidneys of HRS patients, some seen only with electron microscopy.^[16,17] This may be due to cellular changes associated with chronic activation of angiotensin-aldosterone system.^[18] It is conceivable that deep vascular constriction of the renal vascular causes subclinical tubular damage to at least a subset of nephron, which is undetectable by urine sodium which is not sensitive enough to detect mild or patchy tubular epithelial damage. This is supported by Cavallin *et al*, who reported that type 1 HRS was not completely functional, but was also associated with renal tubular damage.^[19] The subclinical tubular damage caused an increase in level of uNGAL in HRS patients.

In this study it was found that the uNGAL level in type 1 HRS was significantly higher than type 2 HRS. These results are consistent with the research conducted by El-Bassat *et al* and Fagundez *et al*, but differ from the results obtained by Gungor *et al*.^[10,11,13] More severe and profound renal vasoconstriction in type 1 HRS may be the cause of higher uNGAL values in type 1 HRS compared to type 2 HRS.^[12]

The results of this study are in line with those reported El-Bassat and Gungor that there is a positive correlation between uNGAL and serum creatinine and negative correlation with GFR. This shows that the higher level of uNGAL, the worse kidney function will be. Several previous studies have also reported that an increase in NGAL level precedes an increase in serum creatinine in the event of AKI.^[20,21,22]

The results of the study also showed that there was a positive correlation between uNGAL and the degree of liver disease severity as indicated by the Child-pugh score. Based on the pathophysiology, HRS occurs due to the presence of portal hypertension which results in a decrease in effective arterial volume so that the body responds with activation of the neurohumoral system (RAAS, SNS, ADH) which will cause renal vasoconstriction

resulting in HRS.^[23-27] So that HRS usually occurs in conditions of severe liver disease, a study by Garcia-Tsao *et al.* reported that HRS occurs in advanced liver disease with high mean child-pugh scores.^[28]

CONCLUSION

Urinary NGAL levels in type 1 HRS was significantly higher than type 2 HRS, possibly because of more severe renal vasoconstriction occurring in type 1 HRS. There is a positive correlation with moderate strength between uNGAL levels with serum creatinine and child-pugh scores, as well as a negative correlation with weak strength between uNGAL levels and GFR in HRS patients.

ACKNOWLEDGEMENT

The authors gratefully acknowledge that the present research is supported by the Ministry of Research and Technology and Higher Education Republic of Indonesia. The support is under the research grant DRPM (Direktorat Riset dan Pengabdian Masyarakat).

Conflict of Interest: None

Source of Funding: None

Ethical Approval: Approved

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How to cite this article: Panggabean LO, Siregar GA, Lindarto D et.al. Urinary neutrophil gelatinase-associated lipocalin in type 1 and type 2 hepatorenal syndrome and its correlation with kidney function. *International Journal of Research and Review*. 2021; 8(7): 359-364. DOI: <https://doi.org/10.52403/ijrr.20210750>
