ORIGINAL ARTICLE

CORRELATION OF URINARY NEUTROPHIL GELATINASE ASSOCIATED LIPOCALIN WITH ERYTHROPOIETIN IN ACUTE KIDNEY INJURY

Aamir Asmatullah, Ayesha Sardar*, Maham Khalid, Rashid Mahmood**, Aslam Khan Mohmand, Fatima Munir*****

Department of Physiology, Pak International Medical College, *Rehman College of Dentistry, **Rehman Medical College, ***M. Phil Scholar, Department of Physiology, Khyber Medical University, Peshawar, Pakistan

Background: Neutrophil gelatinase-associated lipocalin (NGAL) and erythropoietin (EPO) are emerging biomarkers in acute kidney injury (AKI). While both markers are elevated independently in AKI, their correlation has not been explored. This study aimed to evaluate the correlation between urinary NGAL and serum EPO levels in patients with AKI. **Methods:** This comparative cross-sectional study was conducted at a tertiary care hospital in Peshawar. A total of 106 AKI patients (58 males, 48 females) aged over 18 years were enrolled through purposive sampling. Patients with chronic kidney disease, diabetes mellitus, hypertension, autoimmune diseases, or on long-term medications were excluded. AKI was staged using RIFLE criteria. Urinary NGAL and serum EPO levels were measured and compared across the severity stages. **Results:** Among stage 1 AKI patients, NGAL levels were 126±43 ng/mL in females and 138±37 ng/mL in males (p=0.29), while EPO levels were 82.8±32 mIU/mL in females and 139±74 mIU/mL in males (p=0.002). In stage 2 AKI, NGAL values were 116.1±30 ng/mL in females, 135±36 ng/mL in males (p=0.15), and EPO was 99±65 mIU/mL in females and 102±35 mIU/mL in males (p=0.87). Stage 3 AKI cases had NGAL 117.5±41 ng/mL in females and 126±42 ng/mL in males (p=0.52), and EPO 131±69 mIU/mL in females and 117±57 mIU/mL in males (p=0.50). **Conclusion:** Except in stage 1 AKI, no significant correlation was found between urinary NGAL and serum erythropoietin levels. Disease history and duration, glomerular filtration rate, or lack of serial biomarker measurements may cause results variability.

Keywords: Acute kidney injury, Biomarkers, Erythropoietin, Nephrotoxicity, NGAL, Renal failure

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INTRODUCTION

Acute Kidney Injury (AKI) is a critical clinical syndrome characterized by a rapid decline in glomerular filtration rate, leading to impaired excretion of metabolic waste.[1] It is associated with an increased risk of cardiovascular events, progression to chronic kidney disease, and high mortality.[2] The diagnosis of AKI relies on clinical investigations, primarily serum creatinine levels and urine output, which assess kidney function rather than direct kidney injury.[3] However, these markers have limitations, as they do not predict adverse outcomes, hospital stay, treatment costs, or mortality, nor do they identify the underlying cause of AKI.[4] To improve diagnostic accuracy and management, reliable biomarkers indicative of kidney injury has been identified in blood and urine. These biomarkers, when used alongside conventional markers, enhance early detection and treatment strategies. Erythropoietin (EPO) exhibits renoprotective properties, potentially mitigating AKI progression.[5]

Timely diagnosis and management are essential for optimal AKI prognosis. Serum creatinine, a delayed and nonspecific marker of kidney injury, limits early detection.[6] Neutrophil gelatinase-associated lipocalin (NGAL), a glycoprotein of the Lipocalin 2 family, is an early and sensitive biomarker, significantly upregulated in ischemic and nephrotoxic AKI, with detectable levels in blood and urine.[7] NGAL expressed from the tubular epithelial cell and tubular epithelium of the distal nephron after damage, therefore increase NGAL in the urine and serum reflect kidney disease. Following damage to the kidneys, especially the tubular epithelium of the distal nephron, NGAL is released from tubular epithelial cells, leading to elevated levels in both urine and serum.[8] Therefore, increased NGAL concentrations in these fluids are indicative of kidney disease.[9]

Erythropoietin (EPO), induced by hypoxia, exhibits renoprotective properties through anti-apoptotic and regenerative mechanisms.[10,11] There is impaired production of NGAL and EPO in AKI patients in different studies[8,12], however, literature yield no study of its kind to find out the levels of both these markers in the same patients. Correlation between the two markers may enable us to evaluate and treat AKI efficiently. This study investigated the correlation between urinary NGAL and EPO to enhance early AKI diagnosis and management, potentially alleviating patient burden.

METHODOLOGY

This comparative cross-sectional study was conducted after getting approval from the Institutional Research and Ethical Board vide certificate No. DIR/KMU-AS&RB/CU/001747. Ethical approval was obtained from IBMS, Khyber Medical University via diary No. 2022/9298 along with approvals from Hayatabad Medical Complex via diary No. 7413/2022 and Khyber Teaching Hospital via diary No. 4219/2022. Data was collected from Institute of Kidney Diseases, Hayatabad Medical Complex, and Khyber Teaching Hospital, Peshawar.

Keeping the confidence interval of 95%, prevalence of 20% and power of 80, the sample size was calculated as 106. In total about 800 patients were screened, out of which 106 patients fulfilling the criteria were selected for the study. Written informed consent was taken along with identity details from all patients. Adults aged above 18 years with acute onset of symptoms, Azotemia (deranged BUN), and all forms pre-renal, renal, and post-renal cases were included. Chronic Kidney Disease patients, established kidney disease patients due to Diabetes Mellitus, Hypertension, those taking any drugs, patients with autoimmune disorders were excluded.

Anthropometric measurements and blood and urine samples from the patients were collected. The level of NGAL and EPO was determined using Enzyme-linked immunosorbent assay (ELISA).

Data were analysed using SPSS-24. Normality of data was determined. Quantitative data were presented as Mean±SD. Male to female differences were determined through independent sample *t*-test. Correlation between NGAL and EPO was determined through Pearson and Spearmen correlation, and $p \leq 0.05$ was considered as statistically significant.

RESULTS

Table-1 presents baseline data of enrolled patients showing the total sample size, mean age and BMI.

Table-2 shows levels of severity for the 3 stages of AKI. Erythropoietin levels were significantly higher in males in stage 1 AKI than in females (p=0.002). Other parameters showed no significant gender differences.

Table-3 presents correlation analysis among different test parameters. NGAL shows negative correlation with urinary WBCs, while rest of the parameters shows no significant correlation.

Table-1: Demographics of selected individuals

Demographics	Mean values	Female (Mean±SD)	Male (Mean±SD)	*p*
Sample size (n)	106	48	58	
Age (Years)	51.1	48.92±18.51	52.93±20.88	0.3
BMI (Kg/m^2)	23.9	24.50±1.97	23.56±2.23	0.02

Table-2: NGAL and EPO levels according to severity of AKI in males and females

Severity of AKI	Total (n=106) Mean	Male (n=58) (Mean±SD)	Female (n=48) (Mean±SD)	*p*
Stage 1 (n)	45	24	21	
NGAL (ng/mL)	132	138±37	126±43	0.29
EPO (mIU/mL)	112	139±74	83±32	0.002
Stage 2 (n)	27	16	11	
NGAL (ng/mL)	127	135±36	116±30	0.15
EPO (mIU/mL)	100	102±35	99±65	0.87
Stage 3 (n)	34	18	16	
NGAL (ng/mL)	122	126±42	118±41	0.52
EPO (mIU/mL)	124	117±57	131±69	0.50

Table-3: Correlation among different variables

		Age	Level of Severity	BMI	Urinary WBCs	EPO	NGAL
Age	*r*	1	-0.19*	-0.06	-0.08	0.10	0.00
	p		0.04	0.52	0.40	0.28	0.96
Level of Severity	*r*		1	0.04	-0.01	0.07	-0.11
	p			0.63	0.86	0.47	0.24
BMI	*r*			1	0.12	0.11	-0.13
	p				0.20	0.25	0.18
Urinary WBCs	*r*				1	0.10	-0.20
	p					0.29	0.03
EPO	*r*					1	0.12
	p						0.19
NGAL	*r*						1
	p						

DISCUSSION

Our findings showed that males were having high severity of acute kidney injury in all three stages which may be evident by males having more exposure to nephrotoxicity, trauma like road traffic accidents, nephrotoxic radiation work routines along with drugs including smoking and others, while females also subjected to the same as males were found less susceptible to either may be less exposure to trauma or usage of nephrotoxic drugs. Hormones and chromosomes differences in males and females also affect kidneys functions in both genders. These differences affect body response to blood flow, inflammation, and protection against cell damage, especially during kidney injury. Some of these differences are always present, while others happen in response to injury.[13] One study suggests that in mice, the oestrogen helps protect the kidney's filtering barrier during a type of kidney injury caused by reduced blood flow and then its return (ischemia/reperfusion injury).[14] Oestrogen helps widen blood vessels by increasing nitric oxide, while testosterone can reduce this effect. In cases of AKI, females are generally more resistant to inflammation. In some studies, oestrogen has been shown to reduce the activity of immune cells like lymphocytes and neutrophils, which play a key role in causing kidney damage during AKI.[15]

The concentration of urinary white blood cells was found to be associated with low levels of NGAL in our study population which can be a part of multiple

scenarios as in AKI patients, urinary infection is common or can lead to acute tubular necrosis and ultimately AKI. The NGAL seems to be influenced by urinary WBCs and it is reported that the urinary NGAL may be elevated with pyuria because neutrophils contain NGAL. The negative correlation may be in part that urine NGAL can stay elevated up to 7 days after the AKI has developed and levels decrease down after or displacement of the NGAL by the white blood cells.[16]

Erythropoietin levels show no significant association with the level of severity of AKI and with urinary NGAL at any stage of AKI in our study. Previous studies also reported that EPO concentrations cannot discriminate the severity of critical illness in ICU patients.[17] Some studies also showed negative correlation between EPO and NGAL in chronic kidney disease patients.[9]

CONCLUSION

Except in stage 1 AKI, no significant correlation was found between urinary NGAL and serum erythropoietin levels. Erythropoietin may not play a direct role in influencing NGAL levels or *vice versa* in AKI. Disease history and duration, glomerular filtration rate, or lack of serial biomarker measurements may cause results variability.

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Address for Correspondence:

Dr Aamir Asmatullah, Department of Physiology, Pak International Medical College, Hayatabad, Peshawar, Pakistan.
Cell: +92-333-9214955
Email: ask4amir_1@hotmail.com

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Authors approved the draft and are accountable in ensuring that questions related to accuracy or integrity of the work are duly investigated and resolved.

AA: Concept, study design, data analysis and interpretation, drafting, revision
AS: Data analysis and interpretation, drafting
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RM: Concept, study design, critical review
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