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CORRELATION BETWEEN PLASMA NEUTROPHIL GELATINASE-ASSOCIATED LIPOCALIN AND ACUTE KIDNEY INJURY IN ADULT TRAUMA PATIENTS FOLLOWING SEVERE TRAUMA ADMITTED IN INTENSIVE CARE UNIT

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ABSTRACT

Background: Acute kidney injury (AKI) is a common problem in critically ill patients and independently increases morbidity and mortality. Recently, the new biomarker neutrophil gelatinase associated lipocalin (NGAL) has been shown to be superior to creatininefor early detection of renal function impairment.We compared serum NGAL with serum creatinine for early detection of renal dysfunction according to RIFLE criteria.

Methods: Serum creatinine and serum NGAL were measured and glomerular filtration rate (GFR) was estimated within 24 hours of ICU admission. The primary outcome was AKI according to RIFLE criteria within 2^{nd} to 7^{th} day of admission.

Results**:** A total of 260 adult patients from trauma ICU were enrolled in this study. According to RIFLE criteria, 26(10.1%) patients experienced AKI during the first day of ICU admission. The incidence of AKI during the first week of ICU admission was 15% ; (Risk 7.3%, Injury 4.2%, and failure 3.5%) .Development of AKI during the first week of ICU admission was significantly associated with mortality ($p<0.001$). NGAL level more than 150 ng/ml was associated with more risk to have AKI.

Conclusion: Serum NGAl level is a useful and early biomarker for predicting AKI in patients with severe trauma. Serum NGAL level above 150 ng/ml could be considered as a sensitive level for early diagnosis of AKI in trauma patients.

Keywords: Acute kidney injury, trauma, neutrophil gelatinase associated lipocalin,creatinine, Glomerular filtration rate , intensive care unit

INTRODUCTION

One of the most important reasons of mortality in trauma patients admitted to intensive care unit (ICU), is acute kidney injury (AKI) (1, 2) .The incidence of AKI in the intensive care unit is above 25% and carries an overall mortality rate of 50-80% (3). So its detection during the first hours of the admission is critically important (3). AKI might affect 20% to 30% of hospitalized patients; it carries significant costs and is independently associated with increased morbidity and mortality (4). Up to now, the definition of AKI has been based on serum creatinine (5). Where it is classified according to a change from baseline serum creatinine and urine output (risk, injury, failure, loss, end stage renal disease –RIFLE criteria) (1, 5). However this classification has not been reliable enough due to a variety of reasons (reference)

Looking for a promising substitute for creatinine the new biomarker neutrophil gelatinase associated lipocalin (NGAL) has been found (6). A 25 kDa protein member of the lipocalin family, NGAL has recently been proposed as an early marker for AKI, shortly after renal insult and before an increase in serum creatinine (1, 5-7).

The current study was conducted to accomplish two goals: 1) Earlier prediction of AKI in multiple trauma patients after admission to the ICU. 2) Earlier decision making to initiate preventive and therapeutic actions to hinder the progression of AKI.

Methods

In a prospective cohort study we enrolled adult trauma patients who were admitted in the ICU of a trauma referral university-affiliated hospital (Rajaee hospital, Shiraz, Iran). Patients were enrolled within 24 hr of their admission in the ICU from Jan 2009 to Dec 2010.

The study was approved by the institutional review board and informed consent was obtained from patient or their surrogate. Exclusion criteria were patients age less than 18 years , pure neurotrauma or burn ,known history of renal disease and injury severity score (ISS)<15 .

Documented variables were demographics , admission diagnosis , operative status , need to mechanical ventilation , previous medical and drug history , primary and secondary ICU diagnosis and co-morbidities including (Allergy ,Hypertension ,diabetes ,cigarette smoking ,drug abuse).

Besides, need for renal replacement therapy (RRT) , ICU outcomes (mortality ,length of ICU and hospital stay ,days under mechanical ventilation) ,the Acute Physiology and Chronic Health Evaluation (APACHE) IV score, injury severity score (ISS) and acute physiology score (APS) were also recorded.

Blood sample for NGAL 5cc was collected at the first day of admission and freezed at -80 c until analyzed .NGAL was measured in these samples with an Elisa technique (Quantikine ELISA, USA). Serum creatinine (Scr) was measured daily by Jafee colorimetric method. Urine output was measured and documented every hour .All Patients had not baseline Scr before the admission therefore the baseline Scr was estimated by solving the Modification of Diet in Renal Disease (MDRD) equation. In the MDRD equation, we assumed glomerular filtration rate (GFR) based on the lower end of the normal range i.e. 75 cc/min/1.73m2 (9). Weighting the patients was difficult for us; therefore we used a minor modification of the RIFLE urine

output criteria, assuming an average patient weight of 70 kg. This modification has been performed previously and was shown to be associated with meaningful clinical outcomes (9). Based on urine output measured in all patients within first 24 hr (at 6, 12 and 24 hr), we divided the Patients into three groups, urine output <210cc/6hr (Risk), <420cc/12hr (Injury) and <500cc/24hr (Failure). We assumed, 150 ng/ml (10) the cut off value for NGAL concentration for optimal sensitivity and specificity to predict AKI.

Results

The data of a total of 260 trauma patients aged more than 18 years who were admitted in ICU were analyzed. Mean age was 35.2 years with 16.1 years standard deviation. 232(89.2%) of them were male ranged from 18 to 80 years. Mean of the acute physiology and chronic health evaluation IV (APACHE IV) score was 59.7(19-164), mean of acute physiologic score (APS) was 56.9(8-164) and mean of injury severity score (ISS) was 31.9(16-65). Incidence of multiple traumas was 80.8%. (Demographic data are shown in table 1).

Table1: Demographic data of trauma patients admitted in intensive care unit (n=260). Mean (SD)[range]

Age (years)	**35.15(16.17)[18-80]**
Height(cm)	**171.7(6.4)[155-185]**
Male, n (%)	**232 (89.2%)**
APACHE IV	**59.7(21.9) [19-164)**
APS	**56.9(21.1) [8-164]**
ISS	**31.9(9.6) [16-65]**
Mechanical ventilation (day)	**6.5(4.7) [1-35]**
***Duration of admission (day)**	**11.2(7.6) [2-40]**
ICU mortality, n (%)	**52(20%)**
APACHE IV >50 n (%)	**161 (61.9%)**
APS > 50 n (%)	**94 (36.2%)**
ISS> 25 n (%)	**194(74.6%)**
Need to mechanical ventilation; n (%)	**215(82.7%)**
History of co- morbid problems	
HTN n (%)	**30 (11.5%)**
Smoking	**120 (46.2%)**
Drug abuse	**46(17.7%)**
Diabetus	**8(3.1%)**
Nephrotoxic drug	**21(8.1%)**
Multiple trauma	**210 (80.8%)**
Renal replace therapy (n)(%)	**3(1.2%)**

*****Excluding mortality Abbreviations: APACHE =Acute Physiology And Chronic Health Evaluation, APS= Acute Physiology Score, ISS=Injury Severity Score, ICU= Intensive Care Unit, HTN=Hypertension DM=Diabetes mellitus

Collected data for co-morbid conditions revealed: history of hypertension (11.5%),

smoking (46.2%), drug abuse (17.7%), Diabetes mellitus (3.1%) and history of nephrotoxic drug consumption (17.7%).

Mean duration of mechanical ventilation and ICU stay were 6.5 and 11.2 days respectively. Two hundred and fifteen (82.7%) patients underwent mechanical ventilation. Overall ICU mortality rate was 20% (52patients). According to RIFLE criteria, 26(10.1%) patients experienced AKI during the first day of ICU admission and 39(15%) patients (Risk 7.3%, Injury 4.2%, and failure 3.5%) during first week (2^{nd} to 7^{th}) day (table 2). GFR was calculated based on serum creatinine. Development of AKI during the first week of ICU admission was significantly associated with mortality (p<0.001).

Table 2: Renal status based of RIFEL criteria

Renal status	RIFLE 1 Cr n (%)	RIFLE 2-7 Cr n (%)
Normal	234 (90)	221 (85)
Risk	22 (8.5)	19 (7.3)
Injury	3 (1.2)	11(4.2)
Failure	1(0.4)	9 (3.5))

Age, acute physiology and chronic health evaluation IV (APACHE IV), acute physiologic score (APS), injury severity score (ISS), GFR based on first day creatinine and opium addiction were significantly associated with mortality in ICU. The association of the above factors with mortality in ICU was evaluated using binary logistic regression model. Acute physiologic score (APS) (OR=2.6, 95% CI: 1.10-6.38, p<0.001), injury severity score (ISS) (OR=4.02, 95% CI: 1.33-12.11, p<0.001) and GFR based on first day creatinine (OR=2.3, 95% CI: 1.01-5.31, p<0.001) remained significant in the model. Patients with abnormal GFR, APS more than 50 and ISS higher than 25 were at a higher risk of mortality (Table 3).

Table 3: Factors associated with mortality in ICU (univariate and using binary logistic regression model)

Variable	Univariate	Logistic Regression Model	
	P value	P value	Odds Ratio
APACHE	0.003	NS	
APS	<0.001	0.029	2.6
ISS	<0.001	0.013	4.02
GFR based on Creatinine	<0.001	0.046	2.3
Opium Addiction	0.007	NS	
Age	0.017	NS	

Acute physiology and chronic health evaluation (APACHE), acute physiologic score (APS), injury severity score (ISS), GFR based on first day creatinine, opium addiction, mechanical ventilation and level of NGAL were significantly associated with Acute Kidney Injury. (P= 0.012)

Using binary logistic regression analysis association between the factors that were significantly associated with Acute Kidney Injury was investigated. APS (OR=7.3, 95% CI: 2.04-26.36, P<0.001), GFR (OR=8.7, 95% CI: 3.43-22.36, P<0.001), NGAL level (OR=0.35, 95% CI: 0.13-0.95, P<0.012) and mechanical ventilation (OR=0.32, 95% CI: 0.11-092, P<0.006) were significantly associated with AKI. Patients with APS>50 (P < 0.001) and abnormal GFR (P < 0.001) were at a higher risk of AKI. Patients with NGAL level less than 150 ng/ml and those who were not under mechanical ventilation were less likely to have AKI. (Table 4)

Table 4: Factors associated with Acute Kidney Injury from 2nd to 7th day (univariate and using binary logistic regression model)

Variable	Univariate	Logistic Regression Model	
	P value	P value	Odds Ratio
APACHE	<0.001	NS	
APS	<0.001	0.002	7.3
ISS	0.011	NS	
GFR based on Creatinine	<0.001	<0.001	8.7
Opium Addiction	0.022	NS	
NGAL	0.012	0.039	0.35
Mechanical ventilation	0.006	0.035	0.32

Discussion

This research conducted in a referral trauma center in southern of Iran and the aim of study was evaluating the diagnostic value of serum NGAL in the prediction of early AKI in multiple trauma patients who were admitted in surgical intensive care unit. We enrolled only adult patients with severe injury in this study (Mean age= 35.2).

First, several non-renal factors such as age, gender, medication, nutrition status, muscle mass and tubular secretion can influence serum creatinine. Second, in patients undergoing dialysis, serum creatinine measurement is not useful, as it is instantly cleared by all modalities of renal replacement therapy. Third, it has been noticed that in a number of subjects serum creatinine will not raise until 50% of kidney function is lost and creatinine is a late biomarker of reduced glomerular filtration rate (GFR) (3, 5).Furthermore, the progress of the kidney injury cannot be reflected by the initial measurement of serum creatinine since its accumulation always lags behind the insult (5). Considering the shortcomings of creatinine-based methods mentioned above ,the search for a reliable real time marker of AKI is now proved critical(1, 7, 8).In another word ,since a delayed diagnosis of AKI can result in prolonged hospitalization and even excess mortality the

implementation of early renal-protective interventions is essential(1,5,7) .

So what are desirable characteristics of AKI biomarkers?

1) They should be noninvasive, using feasibly accessible samples (blood, urine).

2) They should be rapidly and constantly measurable using a standard assay platform.

3) They should be highly sensitive to early detection and with a wide dynamic range and cutoff values that allow for risk stratification.

4) They should be extremely specific for AKI and enable the identification of AKI subtypes and etiologies.

5) They should demonstrate strong biomarker features on receiver-operating characteristic (ROC) curves (3, 5, 8).

Actually the novelty of our study is evaluating the diagnostic value of serum NGAL in trauma patients. Although other studies have shown this correlation (4, 9, 10), almost all of them enrolled a mixture of patients with medical and surgical problems in their studies(11)and none of them focused on patients with severe traumatic injury separately.

We found a significant correlation between the level of serum NGAL above 150 at the first day of admission and development of AKI in patients with severe trauma injuries. Although previously researchers were able to show the NGAL level more than 400 (11) is significantly diagnostic for AKI but our study revealed that serum NGAL level above 150 could be more sensitive to predict the possibility of developing AKI in trauma patients. Even though this level of NGAL is not very specific to predict AKI but we should notice that trauma patients usually require more radiographic procedures using contrast dyes which can aggravate the susceptibility for AKI. So, we propose an NGAL level above 150 for prediction of development of AKI as it will reduce the false negative results in this group of patients.

This study also revealed that AKI significantly correlates with APS > 50, mechanical ventilation and reduced GFR. Haase, et.al also showed that using NGAL/sCREA can identify approximately 40% more AKI patients comparing to sCREA alone and these patients usually have longer ICU and hospital stay, renal replacement therapy and death compared to the control group in their study. Finally they concluded that serum and urine NGAL showed similar pattern in terms of assessed outcomes in their study(11),our study also is consistent with other studies (12, 13) showed that acute physiology and chronic

health evaluation (APACHE), acute physiologic score (APS), injury severity score (ISS), GFR based on first day creatinine, opium addiction, mechanical ventilation and level of NGAL were strongly associated with development of acute kidney injury.

We didn't follow the changes in NGAL level after the first day of admission but Mishra et.al reported when NGAL is increasing, serum creatinine will increase too, although this increment is independent. This study besides other similar ones revealed that changes in serum creatinine level is a delayed, low-sensitivity indicator for predicting AKI, and even could be a misleading biomarker (4, 10)because it can be affected by many confounding factors (14). In this regard, we consider NGAL level a more-sensitive biomarker for identifying previously undetected AKI in patients with severe traumatic injury.

We found, patients with severe traumatic injury are more likely prone to AKI when their APS is more than 50 ($P<0.012$) and at the same time if they require mechanical ventilation or their GFR is abnormal the risk of AKI will be higher compared to the patients with normal GFR or who doesn't need artificial ventilation ($P<0.001$).This susceptibility could be predicted simply by measuring NGAL level. As our findings showed, patients without mechanical ventilation and NGAL level less than 150 at the first day of ICU admission are less likely to develop AKI (Table 4)

It was expected that APACHE IV more than 50 can significantly increase the risk of AKI ($P<0.001$) in patients with severe traumatic injury. Meanwhile severity of injury (ISS >25) also has a significant role in developing AKI ($P<0.011$).

We couldn't categorize our patients according to their substance abuse habits properly, but our data revealed the relation of AKI in patients with history of opium addiction and severe trauma ($P<0.022$), It needs to be evaluated with a well designed research focusing on these category of patients.

As Haaseet. al suggests, we also recommend inclusion of NGAL as a biomarker for refining a new definition for AKI instead of serum creatinine or urine reduction alone, because the changes in NGAL levels are rapid (hours) but changes in serum creatinine is slow (days)(11). In fact, using NGAL to diagnose subclinical state of AKI will help us to pay more attention to medical condition and making any treatment more likely to succeed.

Some studies have shown that plasma NGAL measurements may be influenced by a number of coexisting conditions like: chronic kidney disease, chronic hypertension, systemic infections, inflammatory conditions, anemia, hypoxia and malignancies (15-17).

Recent reports revealed transient azotemia can increase the risk of death (18)and neutrophil gelatinase-associated lipocalin might help clinicians to identify some of these patients and subsequently reducing this susceptibility by providing early required treatments.

Measuring plasma NGAL level within the first hours after trauma could be highly suggested, sinceas the articles have shown, plasma and urine NGAL concentrations will increase by 10- to 100- fold during the 2 hours following tubular injury(4, 19, 20).

One of the strong points of our study is the large sample size of trauma patients and the fair patient distribution between RIFLE categories, which help us to conclude the ability of NGAL for predicting AKI and as it has been shown in animal studies (21),predicting AKI is very promising to reverse kidney impairment in trauma patients.

Conclusion

Serum NGAl level is a useful and early biomarker for predicting AKI in patients with severe trauma injury. We recommend serum NGAL level above 150 should be considered as a sensitive level for early diagnosis of AKI in trauma patients.

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