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Research Article
Neutrophil Gelatinase Associated Lipocalin (NGAL), an Early Marker for Urinary Tract Infection and Acute Kidney Injury

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Abstract

Background and Objective: Acute kidney injury (AKI) is associated with high morbidity and mortality. Urinary tract infection (UTI) may be associated with sepsis or septic shock, also a major risk factor for diabetics and cause sudden deterioration of renal function. The present study was aimed to examine the microbiological spectrum of UTI and to investigate either serum NGAL could be a promising diagnostic biomarker of AKI following UTI in comparison to other parameters related to renal scaring. **Materials and Methods:** Subjects enrolled in the study comprised 300 with and without diabetes mellitus. Variables such as blood glucose, serum and urine creatinine, urea, hs-CRP and urine Albumin were analyzed by enzymatic methods in Vitros 5.1 FS, HbA1c by HPLC (Bio Rad D10, USA), Serum Neutrophil Gelatinase Associated Lipocalin (NGAL) and Cystatin C by enzyme linked immunosorbent assay kit (Biovendor, USA). Diagnosis of UTI was made from Midstream urine samples of patients with urine culture of $> 10^5$ colony forming units (CFUs) mL^{-1} of a pathogen. Comparison of one time data between three study groups and within Group III was done by One way Analysis of Variance (ANOVA). Correlation among continuous data was performed by the Pearsons correlation coefficients. Area under curve (AUC) was calculated by plotting Receiver operating characteristic curve (ROC). Statistical software program (licensed version 20.0, SPSS, Chicago, IL) was used for all analysis. **Results:** Mean values of biochemical variables such as NGAL, Cystatin C, SCr, hs-CRP and albumin creatinine ratio (ACR) were significant in Group III compared to Groups I and II. Mean$\pm$SD of these biochemical variables within Group III associated with UTI was also observed from '0' day to 14th day. With Pearson's correlation analysis, Scr was significant and positively correlated with NGAL, eGFR, Cystatin C, Urea and hs-CRP. However, NGAL showed strong positive correlation and statistically significant with Scr, eGFR, Urea, hs-CRP, ACR and Cys C. Pair wise comparison of ROC analysis at 95% confidence Interval serum NGAL with an AUC of (0.977, $p = 0.001$), Cystatin C (0.580, $p = 0.41$), SCr (0.756, $p = 0.04$) and ACR (0.654, $p = 0.04$) was observed. **Conclusion:** Physicians should pay attention to UTI patients at risk of AKI (advancing age, DM, upper UTI, afebrile and impaired baseline renal function). Biochemical investigations such as NGAL and hs-CRP play an important role as an adjuvant and surrogate markers in early diagnosis of UTI in parallel to microbiological investigations.

Key words: UTI, AKI, NGAL, hs-CRP, SCr

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Diabetes mellitus (DM) is a heterogeneous group of disorders characterized by variable degrees of insulin resistance, impaired insulin secretion and increased glucose production. DM when associated with kidney disease, it poses a triple threat, not only leads to end stage renal disease (ESRD), but also increases the risk for hospitalization and is one of the major risk factors for development of acute kidney injury (AKI) in hospitalized patients. AKI is a heterogeneous syndrome defined by rapid (hour to days) decline in the glomerular filtration rate (GFR) resulting in the retention of metabolic waste products, including urea and creatinine and dysregulation of fluid, electrolyte and acid base homeostasis[1,2].

The AKI is a common clinical condition associated with a number of adverse outcomes. Timely diagnosis of AKI would allow for earlier intervention and could improve patient outcomes. Infection is the most common and serious complication of AKI, occurring in 50-90% of cases and accounting for up to 75% of deaths. Amongst infections, Urinary tract infections (UTI) are one of the most common triggering conditions for AKI. UTI can be either asymptomatic or symptomatic, characterized by a wide spectrum of symptoms ranging from mild irritative voiding to bacteremia, sepsis, shock or even death. In specific patient groups, urosepsis may show high mortality rates of 25-60%. It is unclear whether this high incidence of infection is due to defect in host immune responses or repeated breaches of mucocutaneous barriers (e.g., intravenous cannulae, mechanical ventilation, bladder catheterization) resulting from therapeutic interventions[3,4].

Number of novel biomarkers of kidney injury has been evaluated for potential roles in the early identification, differential diagnosis and prognosis of AKI. The goal of most AKI biomarker research has been the discovery of a "Kidney troponin", a sensitive and specific early marker of renal injury.

Neutrophil gelatinase associated lipocalin (NGAL), is a member of the lipocalin super family, produced from nephron in response to tubular epithelial damage. It has been identified as one of the earliest and potentially one of the most indicative biomarkers of AKI from a diverse array of conditions and it can differentiate between prerenal and intrinsic causes[5]. Objectives of this study was to assess whether serum NGAL could be a promising diagnostic biomarker of AKI following UTI in comparison to other parameters related to renal scaring and to study the microbiological spectrum of UTI.

MATERIALS AND METHODS

The study was an observational case control study conducted in the medicine unit of R.L Jalappa Hospital a tertiary care referral hospital attached to Sri Devaraj Urs Medical College, a Constituent of Sri Devaraj Urs Academy of Higher Education and Research, South India. A total of 300 subjects were studied during the nine month period. Written informed consent was obtained from all the study subjects. History, duration of diabetes and clinical examinations were recorded in all patients at admission. Diabetes was categorized based on WHO criteria[6].

Diagnosis of UTI was made from Midstream urine samples of patients with urine culture of $>10^5$ colony forming units (CFUs) mL^{-1} of a pathogen. Staphylococcus aureus was considered to be significant regardless of the number of CFUs[7].

Subjects with clinically proven diabetes aged >45 years with history of diabetes more than 5 years, irrespective of treatment (oral drugs and/or insulin therapy) were considered as cases and those without history of diabetes mellitus were included as controls in this study. Patients with DM preexposed to radio contrast induced nephrotoxicity, patients with hepatobiliary disorders leading to proteinuria/albuminuria, Gestational diabetes mellitus and patients already diagnosed with Diabetic nephropathy were excluded in this study.

Sampling method:

Group I : Patient attendees who are free from diabetes mellitus were investigated and the subjects whose biochemical investigations were within biological reference range and clinically proven healthy were included in the study

Group II : One in every fifth patient attending to the Medicine outpatient department with history of diabetes was selected

Group III : Patients admitted in Intensive Care units/Medical Intensive Care Units with history of T2DM, AKI and UTI were categorized into Group III
AKI was defined based on RIFLE criteria[8]

Blood sample was collected in the plain tubes after 8 h of overnight fasting, Fasting and Post prandial blood Glucose (FBG) was analyzed by Glucose Oxidase Peroxidase method (GOD-POD), Blood Urea, Serum and urine creatinine, urine albumin and hs-CRP by enzymatic methods, all these parameters were analyzed in Dry Chemistry Auto Analyzer

Vitros 5.1FS. HbA1c analyzed by HPLC (BIO-RAD D10), concentration of serum NGAL and Cystatin C were estimated by ELISA method (Biovendor, USA), Urinary albumin-creatinine ratio (ACR) was calculated by dividing the urinary albumin concentration in milligram by the urinary creatinine concentration in gram and eGFR by MDRD equation.

Statistical analysis: Comparison of one time data between three study groups and within Group III was done by One Way Analysis of Variance (ANOVA). Mann-Whitney U test was performed for non-normally distributed variables. Correlation among continuous data was performed by the Pearsons correlation coefficients. Chi-square test was done for non-parametric variables.

Receiver operating characteristic curve (ROC) analysis was done to predict better biomarker.

All tests were two-tailed and a $p \leq 0.05$ was considered as statistical significance. SPSS statistical software program (licensed version 20.0, SPSS, Chicago, IL) was used for all analysis.

RESULTS

This was an observational study, conducted in the patients admitted in Medicine Intensive Care units (MICU)/Intensive Care Units (ICU) of a tertiary care hospital over a period of nine months.

Among 200 diabetic patients 138 (69%) were females and 62 (31%) were males. In our study we observed 112 subjects had pus cells in the urine of which 63 had insignificant colony count (Fig. 1). We also observed females with diabetes and AKI had a higher prevalence of UTI compared to men (χ^2 = 9.572, p = 0.003) and statistically significant (Fig. 2).

As shown in Fig. 3, ROC analyses were performed with pair wise comparison of the study markers to define the diagnostic profile of Serum NGAL, Cys C, Scr and ACR. It was observed serum NGAL at 95% confidence Interval, 0.948-1.005 (AUC = 0.977, p = 0.001) Cystatin C at 95% confidence Interval, 0.462-0.699 (AUC = 0.580, p = 0.41), SCr at 95% confidence Interval, 0.665-0.847 (AUC = 0.756, p = 0.04) and ACR 95% confidence Interval, 0.513-0.796 (AUC = 0.654, p = 0.04).

DISCUSSION

UTI where mainly affects the lower urinary tract was known as cystitis and if upper urinary tract it was termed as pyelonephritis [9].

UTI commonly affects elderly people. Around 20% women and 10% men aged 65 years or older were recorded to had bacteriuria[10]. In this study, 60% (180/300) of the cases were over 60 years old (Table 1). It was documented that multiple age related changes such as cell-mediated immunity recession, bladder defenses alteration due to obstructive uropathy, neurogenic dysfunction, bacterial receptivity intensification or uroepithelial cells, contamination due to fecal and urinary incontinence, urethral instrumentation, catheterization and antibacterial factors reduction in prostate and vagina associated with changes in zinc levels, pH and hormones contribute to the risk associated with UTI in elderly[11].

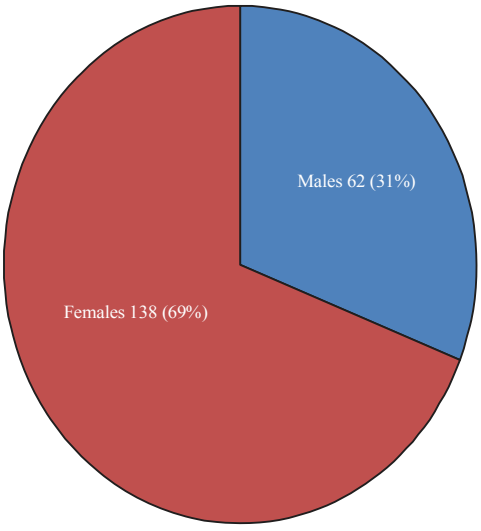


Fig. 1: Gender distribution of patients with diabetes

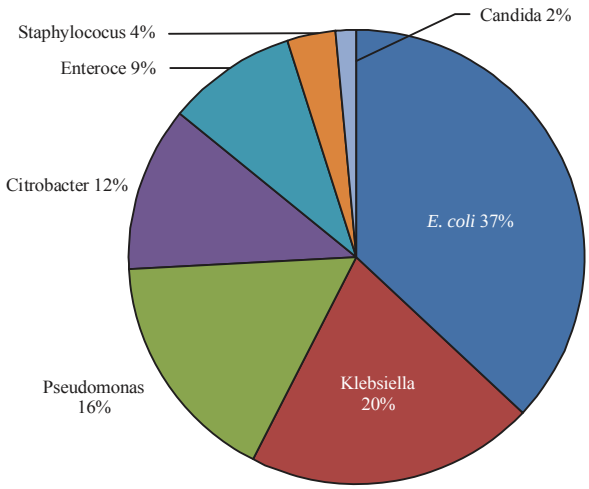
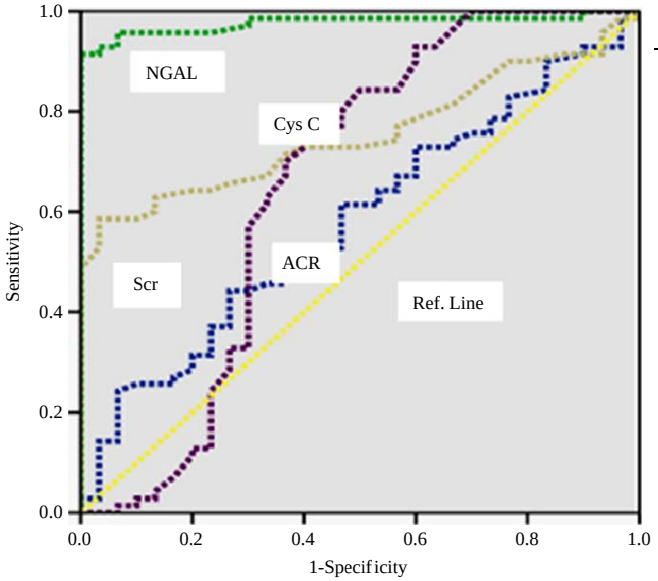


Fig. 2: Distribution of Microorganisms causing UTI in diabetic patients with and without AKI



Variables	Area under Curve (AUC)
NGAL	0.977
CysC	0.580
SCr	0.756
ACR	0.654

Fig. 3: ROC of Serum NGAL, Cys C, SCr and Urine ACR

Table 1: Characteristics of the 300 study subjects

Parameters	Group I Menu±SD	Group II Menu±SD	Group III Menu±SD	ANOVA F-value	p-value
Age (Years)	63.00±9.43	62.00±7.27	62.12±7.12	12.13	0.14
BMI (Kg m^{-2})	22.12±3.13	24.22±3.45	24.62±4.09	38.32	0.072
SBP (mmHg)	120.00±4.32	124.00±5.62	124.00±5.04	1.38	0.112
DBP(mmHg)	82.00±4.62	82.00±3.91	80.00±4.21	2.89	0.410
FBS (mg dL^{-1})	96.60±18.34	197.99±28.91	252.91±35.18	10.16	0.001**
PPBS (mg dL^{-1})	115.21±10.52	205.74±14.27	397.72±51.72	9.65	0.03*
HbA1c (%)	5.91±1.47	10.06±3.55	12.98±3.18	7.33	0.001**
NGAL (ng mL^{-1})	96.42±18.36	156.00±12.35	236.00±35.42	389.04	0.03*
Cys C (mg L^{-1})	1.08±1.23	3.73±2.24	5.97±2.53	12.10	0.001**
SCr (mg dL^{-1})	0.60±0.14	0.95±0.51	4.40±0.46	39.55	0.001**
Urea (mg dL^{-1})	38.41±3.42	56.63±4.85	92.26±10.45	34.12	0.001**
hs-CRP (mg L^{-1})	0.82±1.12	1.76±0.45	2.92±0.95	0.074	0.022*
ACR (mg g^{-1})	15.13±5.36	22.85±4.38	262.15±31.45	15.26	0.001**
eGFE (mL min^{-1} 1.73 m^{-2})	119.66±15.46	84.67±16.55	58.78±8.05	0.46	0.03*

*p<0.05 significant, **p<0.001 highly significant, Group I: Non-Diabetes (clinically proven healthy controls), Group II: Diabetes without AKI, Group III: Diabetes with AKI, BMI: Body Mass Index, SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure, FBS: Fasting Blood Sugar, PPBS: Post Prandial Blood Sugar, HbAlc: Glycated Hemoglobin, SCr: Serum Creatinine, NGAL: Neutrophil Gelatinase Associated Lipocalin, Cys C: Cystatin C, hs-CRP, High Sensitive C-reactive protein, ACR: Albumin Creatinine Ratio

Around 60% of diabetic patients were known to have an increased risk of UTI. UTI in diabetes involved the upper tract in 80% and are more prone to develop complications. Changes in host defense mechanisms, presence of diabetic cystopathy and of microvascular disease in the kidneys may play a role in the higher incidence of UTI in diabetic patients[12]. Studies had shown AKI is a common complication of sepsis and septic shock, UTI is one of the common causes of sepsis and lead to abrupt deterioration in renal function[13].

In the present study, mean values of FBS, PPBS and HbA1c was significantly higher in Group III compared to Group I and Group II. Current findings were supported by the study conducted by Tseng CC *et al*. where they observed patients with HbA1c>8.1% had a higher prevalence of upper UTI[14,15]. Occurrence of UTI in diabetes seems to be related to poor glycemic control and higher glucose concentrations in urine which may promote the growth of pathogenic bacteria. However, studies conducted by Schmitt JK *et al*. and Boyko EJ *et al*. noted that there was no statistical significant association between HbA1c and UTI and HbA1c may serve as a proxy for glycosuria and risk of UTI among diabetic patients, they also stated that sodium glucose co-transporter 2 inhibitors increase glycosuria and were not found to increase the rate of UTI[16,17].

Study done by Park *et al*.[18]. observed that high renal parenchymal glucose levels create a favorable environment

Table 2: Mean and SD of Biochemical Parameters within Group III (T2DM with AKI) Secondary to Urinary Tract Infection

	Number of days			
Variables	0-4	5-9	10-14	p-value
NGAL (ng mL^{-1})	184.00±18.51	210.00±25.16	174.00±19.18	0.012*
Cys C (ng mL^{-1})	5.27±2.53	6.23±2.89	5.12±2.11	0.032*
Ser (mg dL^{-1})	5.80±1.16	5.20±1.14	5.10±1.12	0.021*
hs-CRP (mg dL^{-1})	2.91±0.95	2.35±1.24	1.91±0.19	0.042*
ACR (mg g^{-1})	260.15±30.44	254.08±30.12	250.11±30.04	0.05*
eGFR (mL min^{-1} .1.73 m^{-2})	58.78±8.05	59.01±8.11	59.93±8.91	0.31

*p<0.05 considered as significant

Table 3: Correlation of Scr and NGAL with other biochemical variables in Group III (T2DM patients with AKI) secondary to UTI

	Group III T2DM with AKI (UTI)	
Variables	r-value	p-value
Correlation with Scr		
NGAL	0.613	0.001*
eGFR	0.174	0.021*
Cys C'	0.327	0.004*
Urea	0.425	0.002*
h-CRP	0.221	0.04*
ACR	0.057	0.413
Correlation with NGAL		
Scr	0.613	0.001*
eGFR	0.571	0.001*
Cys C'	0.326	0.031*
Urea	0.523	0.003*
h-CRP	0.525	0.001*
ACR	0.424	0.001

*p<0.05 considered as significant *p<0.05 considered as significant

for the growth and multiplication of microorganisms, which might be one of the precipitating factors of pyelonephritis. Various impairments in the immune system include humoral, cellular and innate immunity may contribute in the pathogenesis of UTI in diabetic patients.

In the present study, it was observed that concentration of serum NGAL was significantly higher at 5-9 days and decreased at 10-14 days phase after antibiotic treatment compared to levels before treatment (Table 2). NGAL is a 25 kDa protein originally purified from human neutrophils. It was considered a specific marker of neutrophil activity and a strong bacteriostatic agent, as it involved the antibacterial iron-depletion strategy of the innate immune system. NGAL was expressed at low levels in normal organs and increases in injured epithelia, including in the lung, colon and especially in the kidney. The levels of NGAL were not raised in healthy full-term newborns at birth, but neutrophils from newborns, even premature infants, have been able to rapidly release NGAL upon bacterial or fungal stimulation *in vivo*. A hospitalized subject with symptoms and signs of acute infections, NGAL was notably increased at admittance with bacterial infections than viral infection, suggesting that NGAL could be a valuable diagnostic marker for differentiating between acute bacterial and viral infections[19].

In this study the purpose of renal parameters was to detect longer duration of inflammation which was analyzed by ROC curve analysis. We observed AUC of Cys C was 0.580 which was not significant compared to ACR (0.654), Scr (0.756) and NGAL (0.977). According to ROC analysis, serum levels of Cys C, Scr and ACR were predictive for the presence of APN in diabetic patients with UTI, Therefore, increased NGAL and Scr in blood and ACR in urine may suggest the presence of APN in subjects with diabetes and AKI (Table 3).

Among these variables, increased serum NGAL could be the most important sensitive and direct biomarker for detecting APN and monitoring the treatment response of diabetes with AKI patients. We also found NGAL showed significant strong positive correlation with other markers such as Scr, eGFR, Cys C, urea, hs-CRP and ACR. In accordance with our findings, Ichino *et al.*[20] observed increased concentration of renal NGAL mRNA and protein levels Yilmaz *et al.*[21]. also demonstrated NGAL levels were higher in UTI group than the control group. However, Piccoli *et al.*[22]. reported conflicting data for using NGAL levels to detect APN in 50 adult patients. While no statistical difference in urine NGAL levels was documented between patients with and without APN, the median urine NGAL tended to be higher in patients with magnetic resonance-proven APN.

As it was known, NGAL is an emerging and excellent urine and plasma biomarker for early prediction, monitoring and determining the prognosis of AKI in several common clinical scenarios. It is transiently expressed in developing nephrons and induces the mesenchymal-epithelial transition, leading to the conversion of metanephric tissue into the glomeruli and proximal renal tubules. NGAL also appears to play an important role in the repair and regeneration of kidney tubule cells after AKI. Study done by Seibert *et al.*[23] and Piccoli *et al.*[24] also revealed both AKI and APN are characterized by acute, mostly tubulointerstitial kidney damage, although the damage is diffuse in AKI and limited to a section of the renal parenchyma in APN.

CONCLUSION FUTURE RECOMMENDATIONS

In this study it was observed hs-CRP levels were higher in diabetics followed by diabetes with AKI patients, increased levels of serum hs-CRP have been suggested to be an indicator for low grade systemic inflammation in several disorders, such as cardiovascular disease and diabetes mellitus. We propose elevated hs-CRP levels could be a new non-invasive candidate biomarker and may be beneficial for follow up of patients with UTI for chronic renal inflammation or disease progression.

These findings may integrate into the current management as for routine clinical use of NGAL and hs-CRP as a surrogate marker with other biochemical variables, where increased serum NGAL concentration may help in differentiating APN from a lower UTI in patients with UTIs. In patients with doubtful UTI elevated NGAL may help for early initiation of treatment and evaluation.

SIGNIFICANCE STATEMENTS

Strengths of this study include NGAL as the most promising biomarker for AKI, estimated hs-CRP as a marker of systemic inflammation. Since, Group III subjects follow up was done for over a period of 14 days, it was also observed that Serum NGAL correlated well with hs-CRP, Scr and ACR and has highest AUC compared to Cys C, Scr and ACR in patients with UTI.

Limitation of present study, this study was single centered, need to be validated in a larger multicenter prospective study with different age groups. However, despite these limitations findings of our study suggest early measurement of Serum NGAL levels could be useful for identifying renal parenchymal involvement in symptomatic UTIs. NGAL level may also be a useful tool for predicting treatment response of diabetic patients with UTI and AKI mainly in females, as we observed that there was a female preponderance of UTI compared to men.

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