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Significance of combined Use of Neutrophil Gelatinase-Associated Lipocalin and Natriuretic Peptides for Diagnosis and Follow up of Patients with Chronic Kidney Diseases

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Abstract

Background

Natriuretic peptides (NPs), are a structurally related group of hormones or paracrine factors sharing a common function of increasing renal sodium excretion and, consequently, reducing expanded extracellular volume. The principal NPs in clinical use are NT-pro BNP and NT-pro CNP. There is some evidence that using NPs tests in emergency departments could replace many expensive tests, and shorten the hospital stay and cost of treatment.
Objectives: To identify the significance of using NPs and NGAL for the diagnosis and/or follow-up of subjects with chronic kidney diseases (CKD) in comparison with routinely used markers.

Material and methods

This study involved 78 participants: 38 controls (seemingly healthy normal adults) and 40 CKD patients. They were 50.6 9.3 and 52.4 8.8 years old on average, respectively. NPs and NGAL were assessed by enzyme-linked immunosorbent assays, while assessments of renal function and serum electrolytes were performed by conventional available laboratory procedures.

Results

There was a significant increase in NT-pro CPN, NT-pro BNP, and NGAL in the patient group compared to the control group. To discriminate between CKD and controls the ROC curve revealed excellent sensitivity and specificity after using the NGAL (90% and 100%, respectively; at a 78.5 ng/ml cutoff value). The sensitivity of NT-pro CNP and specificity were 82% and 90%, respectively, at a 4.95 pg/ml limit value. While the sensitivity of NT-pro BNP and specificity were 72% and 68%, respectively at a 565 pg/ml threshold.
Significant positive direct correlations existed between serum creatinine with each of NGAL and NT-pro BNP.

Conclusion

The combined clinical use of the NPs and NGAL, as adjuvant biomarkers for the CKD diagnosis and follow-up revealed a very good sensitivity and specificity.

Keywords

Natriuretic peptides, Neutrophil gelatin-associated lipocalin, chronic kidney diseases, renal failure.

Chronic kidney disease is defined as reduced kidney function which lasts for many months starting from mild kidney damage to the end-stage disease[2] Its prevalence is about 15% in developed countries[3]. Acute kidney injury i.e. (AKI) together with chronic kidney disease i.e. (CKD) are situations that considerably increase morbidity and/or mortality. Many markers for both types have been reported [4] of which the most common was serum creatinine, which was criticized to lack high predictive value, because of the delay in its rise which may cause difficulties in the identification of early stages of renal parenchymal damage, and that will affect the selection of therapeutic intervention.[5] The heterogeneity and complexity of renal pathology make the diagnosis of acute or chronic kidney damage somewhat challenging. This has led to the development of many other biomarkers in this field. [6] Among these renal biomarkers are the natriuretic peptides and neutrophil gelatinase-associated lipocalin (NGAL). [7]

The natriuretic peptide (NP) is a term applied to a group of related structures having in common the ability to stimulate the excretion of sodium by the kidneys. Human NPs are a complex system of three NPs called atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), as well as C-type natriuretic peptide (CNP) [8]. They have been found to control heart disease along with blood pressure, and are additionally renin-angiotensin-aldosterone system antagonists.

Along with their antimitogenic effects and inhibition of heart hypertrophy and fibrosis, they also play critical roles in endothelial cell function, immunity, cartilage formation, and mitochondrial biogenesis.[9,10] Additionally, CNP was regarded as an autocrine and paracrine mediator in other recent studies. It controls crucial cardiovascular system processes and is derived from heart muscle cells, fibroblasts, and endothelial cells.[11,12]

The extracellular matrix, renal tubules, podocytes, and glomeruli all produce CNP. Due to their ability to act as anti-inflammatory, antifibrotic, and vasodilator agents, they are thought to protect the kidney system.[13,14] Estimated glomerular filtration rate (eGFR) decline was predicted annually based on the baseline level of BNP.[15] A neutrophil gelatinase-associated lipocalin (NGAL) is a 25 kDa protein that has been utilized clinically as a marker for chronic kidney disease or CKD. [15] The urinary NGAL has been considered important in differentiating and managing acute kidney ischemia of liver cirrhosis [16]. NGAL may also play a part in kidney development and kidney injury-related tubular regeneration. [15]

The current study aimed to provide insight into the diagnostic usefulness of using several NPs in combination with NGAL as distinct markers for CKD.

Material and methods

Study Participants

The current study comprised 78 contributors in all. Forty patients with chronic kidney diseases (CKD) as approved by clinical examination and laboratory tests, comprised 26 males and 14 females. Their age was 50.63±9.25 (mean ±SD) years. The duration of CKD ranged from 1.5 – 3 years. None of them had been on dialysis. A total of 38 healthy individuals, 17 men, and 21 women, ranging in age from 52.42 to 8.81 (mean±SD) years, were used in the study as controls. Most of those participants were blood donors.

Demographic characteristics including age and sex, weight, and height were recorded for all participants.

Blood samples were drained from each participant after an overnight fast for measurement of NT-pro BNP, NT-pro CNP, and NGAL by enzyme immunosorbent, ELISA technique, All Kits were purchased from Sunlong Biotech, China. Other tests involving urea, creatinine, and electrolytes (Na^+, K^+, and Ca^{++}) were done on all patients and controls by routine laboratory tests.

Statistical Investigation

To conduct statistical examinations, SPSS software version-25 (USA) was applied. The mean and standard deviation (SD) of continuous data were reported, and ANOVA was performed with the least significant

difference (LSD) as a post hoc analysis. Categorical parameters were presented in the text as percentages/numbers, and a Chi-square correlation was tested to assess them. To distinguish between people with CKD and healthy controls, the effectiveness of NGAL, TN-proCNP, and TN-pro-BNP was assessed using the receiver operating characteristic curve (ROC). When the p-value was less than 0.05, it was concluded that there was a statistically significant difference.

Ethical approval:

The ethics outlined in the Declaration of Helsinki were followed during the study's execution. Before the sample was taken, it was done with the patient's verbal consent. According to file number (150), a local ethics committee evaluated and approved the study design, subject data, and permission form on April 14, 2021, in the College of Medicine at Al-Nahrain University.

Results

Features of the population

There was essentially no difference in the mean ages of the two groups of the study. Females were more prevalent in the control groups than in the patients' group (55.26%), although the difference was not statistically significant. But there were substantial disparities in body weight and BMI, (Table 1).

Table 1: Demographic appearances of the included participants

Variables	CKD (n=40)	Controls (n=38)	Significance
Age/years Mean ±SD Range	 50.63±9.25 33-70	 52.42±8.81 38-69	 0.172
Height (cm) Mean ±SD Range	 168.9±5.64 159-179	 165.26±4.31 155-172	 **0.002**
Weight (kg) Mean ±SD Rang	 78.68±9.05 59-97	 87.21±9.08 65-102	 **<0.001**
BMI Mean ±SD Range	 27.55±2.6 21.2-32.37	 32.0±3.75 23.0-38.29	 **<0.001**
Gender Male Female	 26(65%) 14(35%)	 17(44.74%) 21(55.26%)	 0.061

Renal Function Tests

CKD patients' group had significantly higher levels of serum urea and creatinine than their controls, (table 2)

Table- 2: Serum urea and creatinine of the study participants

Variables	CKD (n=40)	Controls (n=38)	Significance
Urea (mg/dl) Mean±SD Range	 126.2±19.4 95-178	 33.79±6.28 22-48	 **<0.001**
Creatinine (mg/dl) Mean ±SD Range	 5.71±1.5 2.8-9.0	 0.84±0.15 0.6-1.2	 **<0.001**

Serum Electrolytes

Even though the serum levels of all included electrolytes (K+, Na+, and Ca++) in both groups were within normal ranges, there was a difference between them. The levels of K+ and Na+ were higher in the CKD group than in the controls, who had higher Ca++ levels, (table 3).

Table-3: Serum electrolytes of the study participants

Variables	CKD (n=40)	Controls (n=38)	Significance
K^+ (mEq/l) Mean±SD Range	 5.26±0.17[a] 5.02-5.66	 4.32±0.36[b] 3.7-5.0	 <0.001
Na^+ (mEq/l) Mean±SD Rang	 141.3±5.81 132-155	 140.3±2.95 136-147	 > 0.05
Ca^{++} (mg/dl) Mean±SD Range	 8.59±0.34[a] 7.93-9.33	 9.47±0.35[b] 7.93-10.0	 <0.001

Serum NGAL and natriuretic peptides in the study population

The CKD patients showed a marked increase in the three markers used in this study, as compared to their control group, (table 4).

Table -4: Plasma levels of NGAL and NPs in the study participants

Variables	CKD (n=40)	Controls (n=38)	Significance
NGAL (ng/ml) Mean ±SD Range	 110.4±42.3 36-195	 37.7±18.4 14-77	 **<0.001**
TN-pro BNP (pg/ml) Mean ±SD Range	 667.1±163 257-978	 102.3±46.5 21.0-343	 **<0.001**
TN-pro CNP (pg/ml) Mean ±SD Rang	 6.53±1.74 2.6-9.0	 2.24±0.82 0.09-6.3	 **<0.001**

Diagnostic Value of NGAL, TN-pro CNP, and TN-pro BNP using ROC analysis for the differentiation between CKD and healthy controls:

The UAC for neutrophil gelatinase-associated lipocalin was 96.2%, 95%CI = 0.922 – 1.0, and a p-value of 0.001. The test's sensitivity and specificity were 90% and 100%, respectively, at the cut-off value of NGAL= 78.5 ng/ml.

The NT-pro CNP UAC was 0.922, 95%CI=0.867-0.977, and p0.001. The test's sensitivity and specificity were 82% and 90% at a cutoff value of NT-pro CNP=4.95 pg/ml.

The NT-pro BNP UAC was 0.985, 95%CI=0.966-1.0, and p0.001. At the NT-pro BNP cutoff value of 449 pg/ml, the test's sensitivity and specificity were 98% and 92%, respectively (Figure 1).

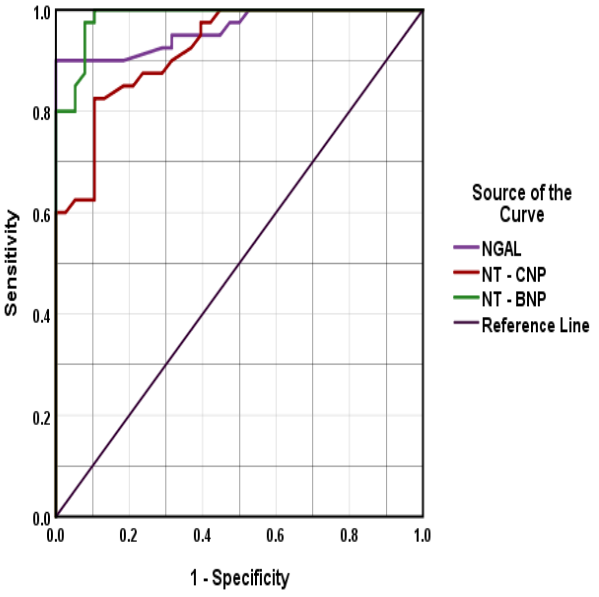

Figure 1: ROC analyses showing the curve of NGAL, NT-proBNP, and NT-proCNP for the differentiation between CKD and controls.

Correlations of NT-proCNP, NT-proBNP, and NGAL with study Variables:

Creatinine displayed a substantial positive direct correlation with both NGAL and NT-BNP (r-0.412, p-0.008, and r-0.313, p-0.049, respectively). Potassium and NT-proCNP have a significant positive correlation (r-0.372, p-0.018).

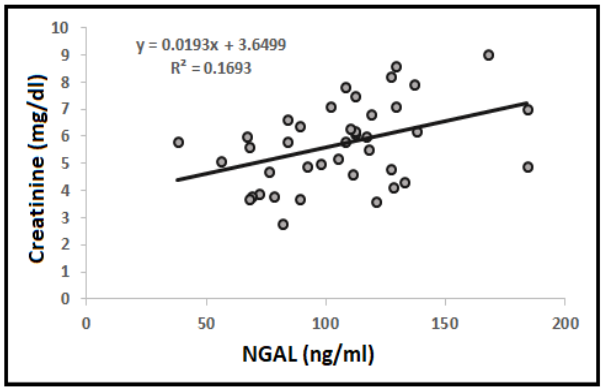

Figure 2: Regression line and scatter plot between NGAL and creatinine in cases with CKD

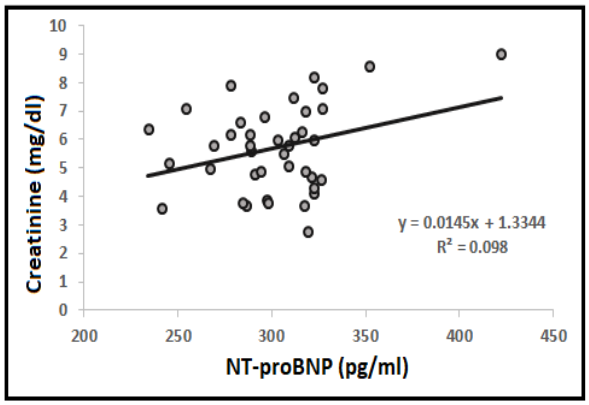

Figure 3: Regression line and scatter plot between NT-proBNP and creatinine in cases with CKD

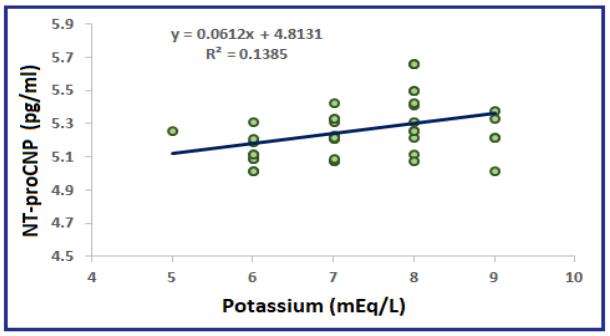

Fig.4: Regression line and scatter plot between NGAL and K^+ in patients with CKD

Discussion

The usefulness of peripheral NGAL as a biomarker in kidney disease was first reported by Devarajan in 2008 and evaluated later on by others,[16,17, 18]. The NPs are markers that have undergone substantial research to be used in the management of heart failure or to recognize subjects at increased risk for cardiotoxicity [19,20,21]. Elevated levels of NPs (BNP and/or CNP) in renal failure were approved by previous works[13,14]. According to some researchers, renal function has a considerably more significant effect on NT-proBNP than it does on BNP.[22,23] The levels of NT-proBNP and BNP are influenced by gender, being higher in females.[23]

Furthermore, the results of the present CKD patients showed significant correlations between serum creatinine and each of NGAL and Nitro-pro BNP. This is in accord with other reports[24]. Hyperkalemia of chronic and acute kidney damage has been attributed to a defect in the renin–aldosterone–angiotensin system and reduced tubular reabsorption of K^+ [25]. No effect of BMI was noted on NGAL[26] and for risk prediction, there was no statistically significant interaction between levels of serum NT-proBNP and measures of BMI.[27]

The ROC curve ROC results indicated reasonable specificity and sensitivity values for both markers to assess the utility of NGAL and NPs in diagnosing CKD. Previous work on NGAL to predict stage 2 CKD found 72.2 % for both sensitivity and specificity at a cut-off value of 98.71ng/mL [24]. Recent work reported sensitivity and specificity for NT-proBNP to be 95% and 80 % respectively at a higher cut-off (1850pg/ml) value for CKD cases not on dialysis[28]. No report on the diagnostic ability of CNP could be found in the literature.

Collectively, these results highly suggest using NT-pro-BNP and NGAL as adjuvant biomarkers in the diagnosis and follow-up of CKD. Further research should investigate the potential therapeutic suppression of these markers in patients with CKD.

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