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Journal of Bioscience and Applied Research

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Elevated serum NAGAL levels were associated with cardiovascular diseases in pediatric chronic kidney disease

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Received 24/2/2020; Accepted 21/3/2020

Abstract

Background: Early cardiovascular disease (CVD) management and prediction have become mandatory in children with chronic kidney disease (CKD). Association between neutrophil gelatinase-associated lipocalin (NGAL) and CVD events in pediatric CKD patients remains unclear. Thus, we aimed to evaluate this relationship and to clarify the association between NGAL serum levels and some CVD related parameters. **Subjects and methods:** A total of 70 children patients with CKD (30 with and 40 without CVD). The patient's data were retrospectively recorded from the medical files of each patient. NGAL serum levels were measured by ELISA commercial kits. Association between different parameters was assessed by the Pearson correlation coefficient. **Results:** NGAL serum levels were significantly (P<0.0001) higher in patients with CVD (2450 (1335-2880) pg/mL) than patients without CVD (371 (285-1363) pg/mL) and healthy controls (295 (166-357) pg/mL). At 1300 pg/mL, NGAL has a good CVD predictive function with high area under curve (AUC=0.871). The sensitivity, specificity, positive predictive value, negative predictive value, and accuracy were 80, 80, 63.2, 90.3 and 80%, respectively. CVD risk increase with elevated NGAL serum levels (>1300 pg/mL) (OR=2.57, 95 CI (1.62-4.09), P<0.0001). Carotid intima thickness was associated with elevated NGAL levels. Both NGAL and carotid intima thickness were significantly correlated with dialysis duration, uric acid, and lipid profile. **Conclusion:** NGAL was associated with CVD events in children with CKD with good predictive value supporting NGAL putative role in CVD pathophysiology. But NGAL in CVD is still in the early stages and future studied needed to evaluate its association with CVD severity.

Keywords: neutrophil gelatinase-associated lipocalin, NGAL, cardiovascular disease, chronic kidney disease.

1. Introduction

In pediatric patients with chronic kidney disease (CKD), cardiovascular disease (CVD) is a leading cause of death **(do Val et al., 2019)**. It is not surprising that the guidelines of the American Heart Association for the reduction of CVD risk in high-risk pediatric patients stratified pediatric CKD in the highest risk category for CVD development **(Mitsnefes, 2012)**. Thus, early CVD prediction and management have become mandatory in children and adolescents with CKD **(El-Gamasy and Mawlana, 2019)**. Moreover, the accurate CVD risk assessment at an early stage would facilitate more focused and aggressive treatment of patients with the goal to decrease event rates **(D'Marco et al., 2015)**.

Many traditional risk factors related to renal function loss (anemia, bone mineral disorders, inflammation, and oxidative stress) are also related to high CVD incidence in CKD patients. Whether biomarkers in CKD patients has been at the core of extensive research to help improve patients at CVD risk identification **(D'Marco et al., 2015; Niizuma et al., 2017)**. Because of various damaging stimuli, neutrophil gelatinase-associated lipocalin (NGAL) is released from injured renal tubular cells. It is known to be one of the most promising biomarkers of impending kidney injury **(Devarajan, 2010)**. As well as renal tubules, the vascular walls in rats responded to various types of damage by overexpressing NGAL protein **(Cruz et al., 2012)**. Evidence suggests that NGAL plays a crucial role in atherosclerosis in plaque instability and vascular remodeling **(Hemdahl et al., 2006; Folkesson et al., 2007)**. Increased NGAL levels have been reported in many CVD conditions, including coronary heart disease, both acute and chronic heart failure and stroke **(Cruz et al., 2012)**. Collectively, these findings provide biological plausibility for NGAL potential role as CVD biomarker in patients with CKD **(Cruz et al., 2012)**.

To date, the relationship between NGAL and CVD events in pediatric CKD patients remains unclear. We, therefore, aimed to evaluate this relationship and to clarify the association between NGAL serum levels and some parameters related to CVD events including dialysis duration, lipid profile and carotid intima-media thickness.

2. Material and methods

2.1. Study population

The present study consisting of children (n=70) aged 4-16 years with CKD (stage 5) maintained on dialysis in the Pediatric Nephrology Unit of Mansoura Children's Hospital, Mansoura University, Mansoura, Egypt. CKD were urological abnormalities (n=35), glomerulonephritis (n=10), pyelonephritis (n=14) and other unknown causes (n=11). The patient's data were retrospectively recorded from the medical files of each patient. There were classified to 40 patients without and 30 with CVD events. All CVD events were confirmed through the participant's medical records. In addition, thirty control subjects were outpatients of the Mansoura Children's Hospital, aged 5–17 years, without any chronic diseases or renal impairment. Informed consent was obtained from the parents of each patient. This study has been performed according to the Helsinki Declaration's ethical guidelines.

2.2. Laboratory and biomarker measurements

Blood samples collected for serum NGAL analysis were centrifuged at 1000 ×g for 20 minutes and stored at −20°C until analysis. Using fresh serum, liver and kidney function tests and lipid profile were all measured on fully automated chemistry analyzer (DIALAB Autolyser 100, Neudorf, Austria) and minerals were determined using blood gas/electrolyte analyzer (Cobas b 121, Roche Diagnostics). NGAL

was measured using ELISA commercial Kits (Boster Biological Technology, Pleasanton, California, USA).

2.3. Statistical analysis

GraphPad Prism and SPSS software were used for data analysis. Non-normally distributed data are shown as median (interquartile range), and normally distributed data are shown as mean ± standard deviation (SD). Univariate analysis of variance (ANOVA) and the Kruskal-Wallis test was used to compare values between groups with normal and skewed variable distribution, respectively. Proportions in groups were compared using the Pearson $\chi 2$ test. $P<0.05$ was considered statistically significant. The Receiver-operating characteristic (ROC) curve was used to evaluate the predictive function of NGAL serum levels for identifying new-onset CVD in hemodialysis children patients. To assess whether any two variables are associated, the Pearson correlation analysis was used.

3. Results

3.1. Characteristics of study populations

Table 1 showed the main demographic and clinical data of hemodialysis children patients with and without CVD and healthy children controls. Patients with CVD were older (P=0.0321) than others. CKD patients (with/without CVD) were associated with significantly elevated serum levels of creatinine, urea, blood urea nitrogen, uric acid, alkaline phosphatase, sodium, potassium, and phosphorus. There was a significant difference in dialysis duration and carotid intima thickness between children patients with CVD events and other patients without CVD and healthy controls. Also, children patients with CVD events were associated with dyslipidemia, elevated serum levels of cholesterol, triglycerides, low-density lipoprotein, and very high-density lipoprotein and decreased serum levels of high-density lipoprotein.

3.2. Elevated NGAL levels and CVD risk

Median serum levels of NGAL were significantly (P<0.0001) higher in patients with CVD (2450 (1335-2880) pg/mL) than who without CVD (371 (285-1363) pg/mL) and healthy controls (295 (166-357) pg/mL) (Figure 1). At 1300 pg/mL, NGAL has a good CVD predictive function with a high area under curve (AUC=0.871; Figure 2). The sensitivity, specificity, positive predictive value, negative predictive value, and accuracy were 80, 80, 63.2, 90.3 and 80%, respectively. This power increased when patients with CVD were discriminated against from healthy controls only (Figure 3) and the mentioned values increased to 80, 100, 100, 83.3 and 90%, respectively. The odds ratio (OR=2.57, 95 CI (1.62-4.09), P<0.0001) for CVD prevalence increased with the increase in NGAL serum levels (>1300 pg/mL).

3.3. Association between NGAL, carotid intima thickness and other parameters

Carotid intima thickness was associated with elevated NGAL levels. Both NGAL and carotid intima thickness were significantly correlated with dialysis duration and serum levels of uric acid, cholesterol, HDL and LDL (Tables 2 and 3).

Table 1. Patients characteristics

Parameter	Healthy	Hemodialysis patients		*P* value
		Without CVD	With CVD	
Age (years)	6.9±2.7	5.9±3.1	8.6±4.8	0.0321
Gender (male/female)	19/11	58/27	39/26	0.579
Creatinine (mg/dL)	0.55±0.1	7.42±3.3	6.67±1.8	<0.0001
Urea (mg/dL)	18.6±3.4	132.6±71.7	90.5±50.2	<0.0001
BUN (mg/dL)	9.4±2.64	70.8±26.1	72.6±25.9	<0.0001
Uric acid (mg/dL)	3.2±0.49	5.4±1.47	6.6±1.34	<0.0001
ALT (U/L)	20.1±1.4	23.3±5.5	22.3±4.99	0.104
AST (U/L)	17.2±3.3	19.2±4.9	18.0±2.9	0.445
Albumin (g/dL)	4.3±0.53	3.88±0.27	3.86±0.27	0.002
Bilirubin (mg/dL)	0.56±0.1	0.57±0.05	0.56±0.15	0.942
Alkaline phosphatase (U/L)	122 (71-203)	460 (184-1524)	626 (269-706)	0.0007
Sodium (mmol/L)	131.7±1.58	137.9±3.65	138.3±3.63	<0.0001
Potassium (mmol/L)	3.95±0.56	4.76±1.18	4.76±1.40	0.0914
Calcium (mg/dL)	9.28±0.38	8.34±1.47	8.27±1.36	0.0222
Phosphorus (mg/dL)	4.18±0.36	5.25±1.17	5.38±1.27	0.0043
Dialysis duration (months)	—	18.56±5.64	30.52±6.06	<0.0001
Carotid intima thickness (mm)	0.440±0.011	0.449±0.005	0.462±0.003	<0.0001
Cholesterol (mg/dL)	111.6±23.4	163.3±46.0	197.4±60.4	<0.0001
Triglycerides (mg/dL)	98 (55-122)	127 (96-159)	136 (109-178)	0.0485
HDL (mg/dL)	58 (48-69)	55 (39-76)	15 (8-27)	<0.0001
LDL (mg/dL)	38 (18-50)	60 (26-106)	106 (92-182)	<0.0001
vLDL (mg/dL)	20 (11-24)	25 (19-31)	30 (24-47)	0.0031

Abbreviations: BUN= blood urea nitrogen; ALT= Alanine aminotransferase; AST= Aspartate aminotransferase; HDL= high density lipoprotein; LDL= low density lipoprotein; vLDL= very high-density lipoprotein. Normally distributed variables were expressed as mean±SD. Non-normally distributed variables were expressed as median (interquartile range). Significant difference was determined using X^2 test, ANOVA and Kruskal-Wallis tests as appropriate. $P<0.05$ was considered significant.

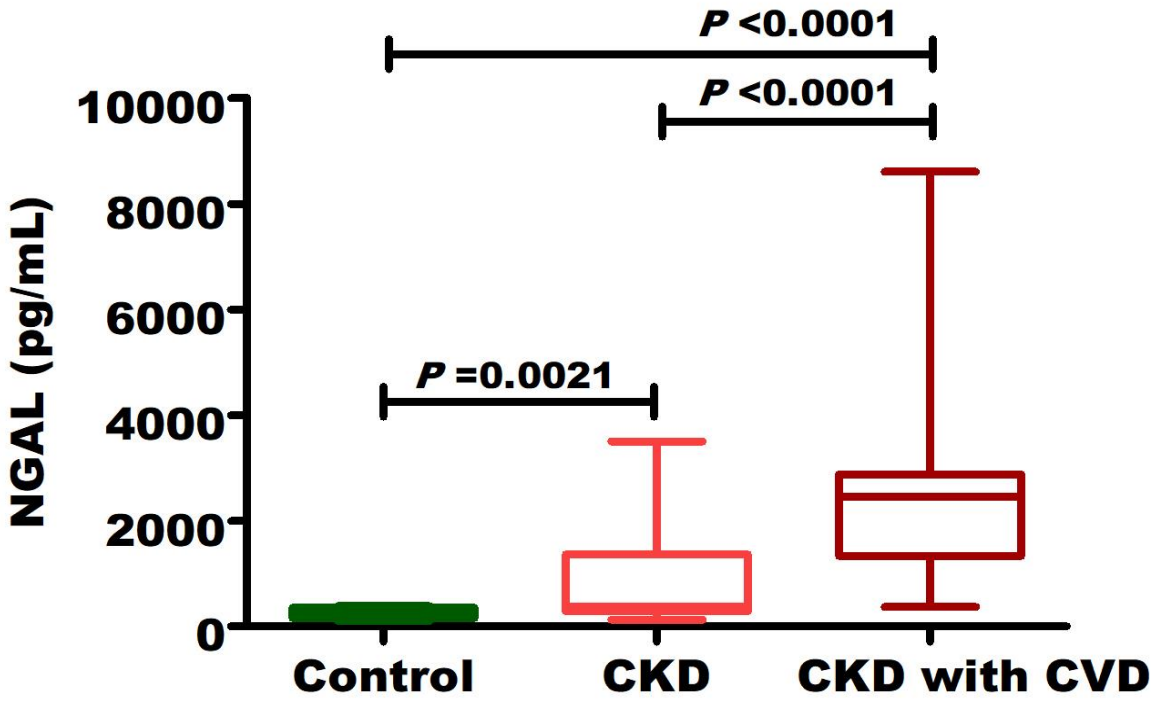

Figure 1. NGAL serum levels of included patients and controls. Significant difference was determined using Kruskal-Wallis tests and LSD as post-hoc comparison method.

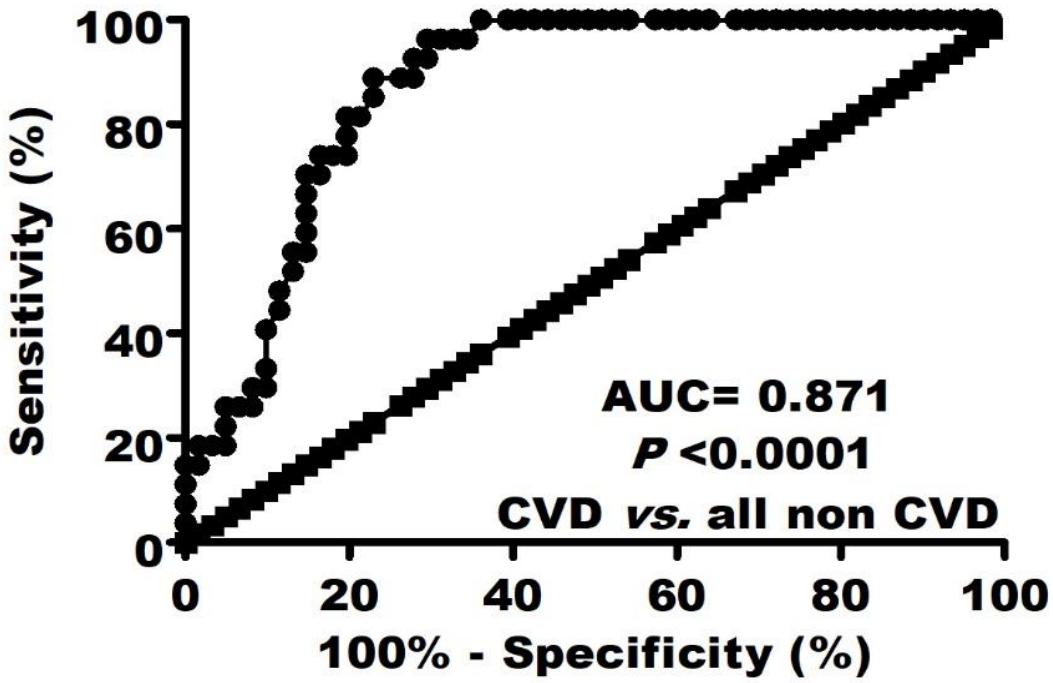

Figure 2. ROC analysis for NGAL to discriminate patients with CVD from all non-CVD individuals.

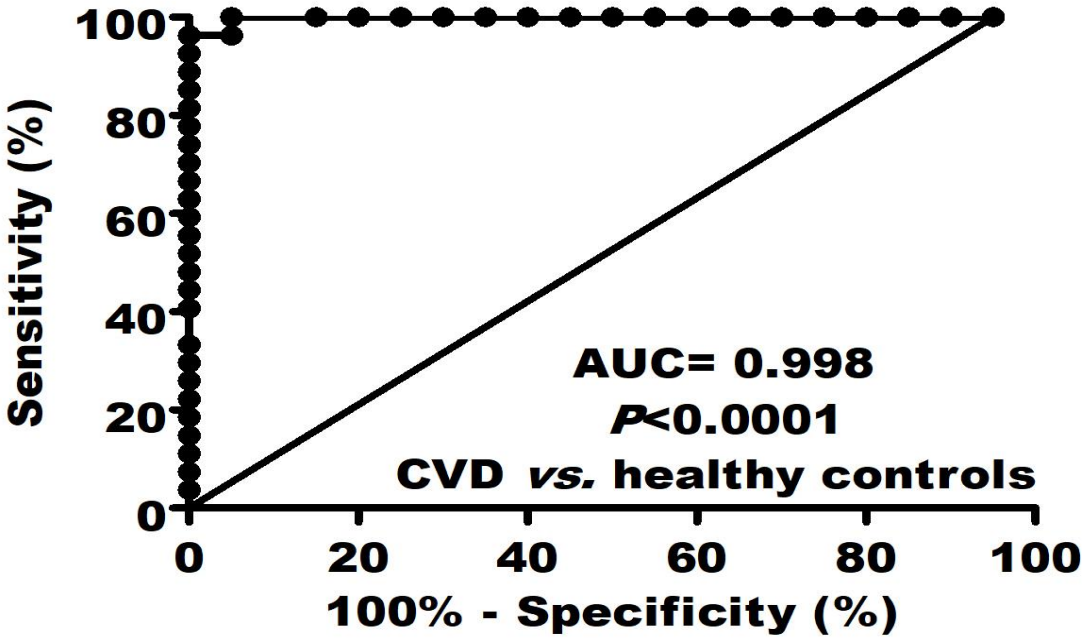

Figure 3. ROC analysis for NGAL to discriminate patients with CVD from healthy controls.

Table 2. Correlation between carotid thickness and other clinical parameters

Factor correlated with carotid thickness	Pearson correlation coefficient (*r*)	*P* value
Creatinine	0.117	0.604
Urea	-0.04	0.889
Blood urea nitrogen	-0.01	0.973
Uric acid	0.663	<0.001
Creatinine	0.117	0.604
ALT	-0.173	0.523
AST	-0.171	0.528
Albumin	0.07	0.673
Bilirubin	-0.03	0.936
Alkaline phosphatase	0.238	0.326
Sodium	0.116	0.616
Potassium	0.08	0.748
Calcium	0.07	0.775
Phosphorus	0.135	0.606
Dialysis duration	0.696	<0.001
Cholesterol	0.328	0.006
Triglycerides	0.08	0.527
HDL	-0.669	<0.001
LDL	0.423	<0.001
vLDL	0.214	0.082
NGAL	0.546	<0.001

Abbreviations: ALT= Alanine aminotransferase; AST= Aspartate aminotransferase; HDL= high density lipoprotein; LDL= low density lipoprotein; vLDL= very high-density lipoprotein.

Table 3. Correlation between NGAL serum levels and other clinical parameters

Factor correlated with NGAL serum levels	Pearson correlation coefficient (*r*)	*P* value
Creatinine	-0.193	0.389
Urea	0.016	0.955
Blood urea nitrogen	0.135	0.632
Uric acid	0.237	0.302
Creatinine	0.117	0.604
ALT	-0.231	0.389
AST	-0.347	0.188
Albumin	-0.081	0.595
Bilirubin	0.105	0.746
Alkaline phosphatase	-0.019	0.939
Sodium	-0.162	0.483
Potassium	-0.194	0.389
Calcium	-0.026	0.911
Phosphorus	-0.112	0.668
Carotid thickness	0.546	<0.001
Dialysis duration	0.437	<0.001
Cholesterol	0.201	0.100
Triglycerides	0.063	0.620
HDL	-0.489	<0.001
LDL	0.285	0.033
vLDL	0.121	0.330

Abbreviations: ALT= Alanine aminotransferase; AST= Aspartate aminotransferase; HDL= high density lipoprotein; LDL= low density lipoprotein; vLDL= very high-density lipoprotein.

4. Discussion

Beyond the glomerular filtration rate (GFR), some studies clearly indicated that NGAL on its own represents a CKD progression marker **(Bolignano et al., 2009b)**. Moreover, beyond the renal physiopathology boundaries, findings made in other studies indicated that this protein may be used in patients with CVD events **(Bolignano et al., 2010)**. NGAL was reported to be independently associated with a higher risk of CVD events in adult patients with CKD. But they recommended further work to determine NGAL utility to improve risk prediction for CVD adverse outcomes **(Zylka et al., 2016; Park et al., 2017)**. As in adults, CKD children patients have a high prevalence of uremia-related and traditional CVD risk factors **(Mitsnefes, 2012)**. In this population, to our knowledge, NGAL did not evaluate as an early marker of CVD.

Here, NGAL serum levels were significantly higher in patients with CVD than patients without CVD and healthy controls. It has good CVD predictive power with good sensitivity, specificity, positive predictive value, negative predictive value and accuracy of 80, 80, 63.2, 90.3 and 80%, respectively. Findings from human tissue and animal studies demonstrate that NGAL is highly expressed in the heart and also expressed in

atherosclerotic plaques and both in failing myocarditis and myocardium **(Cruz et al., 2012)**.

Compared to normal mice, aortas from atherosclerotic mice exhibited higher levels of NGAL mRNA. In the aorta of atherosclerotic mice, hypoxic stress increased NGAL mRNA expression, suggesting that NGAL expression was necessary for the development of myocardial infarction or possibly that NGAL was upregulated as infarction result. In contrast to normal aortas of control mice, in atherosclerotic mice abundant NGAL protein was seen in aortic plaques border regions and lipid core, and co-localized with matrix metalloproteinase (MMP)-9 **(Hemdahl et al., 2006)**. In the acute heart failure rat model, the left coronary artery was ligated **(Yndestad et al., 2009)**, causing transmural infarction of the left ventricular free wall. Rats with heart failure had significantly raised NGAL expression in the left ventricular non-ischemic area, representing development from acute to a chronic stage in this model **(Yndestad et al., 2009)**. In an autoimmune myocarditis rat model, NGAL was strongly expressed in fibroblasts, vascular wall cells, and cardiomyocytes **(Ding et al., 2010)**. In response to IL-1β stimulation, NGAL mRNA and protein expression in vascular smooth muscle cells were also upregulated in an NF-κB -dependent manner **(te Boekhorst et al., 2011)**.

Compared to control (non-failing) human left ventricular tissue, tissue aliquots from failing myocardium (explantation of hearts from end-stage heart failure patients undergoing cardiac transplantation) exhibited strong NGAL immuno-staining, with some immunoreactivity also seen in endothelial cells and vascular smooth muscle **(Yndestad et al., 2009)**. Also, NGAL was detected in leukocytes, fibroblastoid cells, vascular wall cells and cardiomyocytes in human hearts with myocarditis **(Ding et al., 2010)**.

Moreover, many clinical studies reported the role of NGAL in different CVD events. Compared with control subjects, chronic heart failure patients have been reported to have significantly elevated levels of both urine and serum NGAL **(Damman et al., 2008; Bolignano et al., 2009a; Poniatowski et al., 2009; Yndestad et al., 2009)**. Both serum and urine NGAL levels correlated with various renal function indices including GFR, urinary albumin, creatinine, blood urea nitrogen and cystatin C **(Shrestha et al., 2011)**. Similar to findings seen in chronic heart failure, serum NGAL also appeared to correlate with renal functions in acute heart failure **(Aghel et al., 2010; Maisel et al., 2011)** and coronary heart disease **(Choi et al., 2008; Zografos et al., 2009)**.

In this study, carotid intima thickness was associated with elevated NGAL levels and both of them were significantly correlated with some CVD related risks such as dialysis duration and elevated serum levels of uric acid, cholesterol, HDL and LDL. In the cerebrovascular disease rats' model, NGAL was highly induced in the intima after balloon injury to the common carotid artery **(Bu et al., 2006)**. NGAL was also reported to be significantly correlated to dyslipidemia **(Dolapoglu and Beketaev, 2015)** and uric acid **(Tomczak et al., 2013)**.

Conclusion

NGAL was reported to be associated with CVD events in children with CKD. It has a good predictive value supporting NGAL putative role in CVD pathophysiology. But NGAL in CVD is still in the early stages and future studied needed to evaluate NGAL association with the severity of CVD.

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