Original Article

Investigational Biomarkers in Patients with Chronic Renal Failure

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ABSTRACT

Background: Several studies showed that urinary concentration of neutrophil gelatinase-associated lipocalin (NGAL) increased in patients with chronic heart failure and mildly reduced glomerular filtration rate (GFR). **Objective:** To compare the NGAL levels between patients with high N-terminal pro-brain natriuretic peptide (NT-proBNP) levels and those with low NT-proBNP levels. **Methods**: In this case control study, we estimated the GFR, NT-proBNP, NGAL and fibroblast growth factor 23 (FGF-23) levels in twenty peritoneal dialysis patients, along with twenty age- and sex-matched healthy controls. Therefore, in patients with NT-proBNP levels higher than 1000 pg/mL (hypervolemic patients) and in patients with NT-proBNP lower than 1000 pg/mL (normovolemic patients), serum NGAL and parameters of echocardiography were evaluated at the beginning of the study and again after three months. **Results**: In this study, patients' serum NT-proBNP levels, NGAL and FGF23 were significantly higher than in the healthy control group ($p < 0.05$). There were no significant differences between hypervolemic patients and normovolemic patients with regard to age, sex, body mass index, serum creatinine, parathyroid hormone, blood urea nitrogen (BUN), urine volume, GFR and left ventricular end-diastolic diameter, left atrial diameter or aortic root size measured by echocardiographic evaluation at the beginning and at the second measurement ($p > 0.05$). Plasma NT-proBNP levels were markedly greater in hypervolemic patients (13323.3±11235.9 pg/mL) than those of normovolemic patients (456.1±266.7 pg/mL) ($p < 0.05$). In hypervolemic patients, there was a significant decrease in NT-proBNP levels at the second measurement (from 13323.3±11235.9 pg/mL to 9667.3±9483.2 pg/mL) ($p < 0.05$). However, no significant changes were detected in serum NGAL levels, FGF23 or GFR. In normovolemic patients, serum NT-proBNP, NGAL, FGF23 and GFR did not change ($p > 0.05$). **Conclusion:** Serum NT-proBNP, NGAL and FGF23 levels are significantly higher in dialysis patients. This further indicates the need for a more comprehensive study of hypervolemia and the reduction of NGAL and residual renal function, which we often encounter in patients with renal insufficiency.

Key words: NGAL, NT-proBNP, FGF23.

Neutrophil gelatinase-associated lipocalin (NGAL) is a member of the lipocalin family of proteins, and it is secreted from kidney tubule cells at low concentrations. Many studies have shown that NGAL is produced in the kidneys after ischemic or nephrotoxic damage [1–4]. The risk of cardiovascular morbidity and mortality in patients with chronic renal failure (CRF) is much higher than in the general population [5–7]. High levels of fibroblast growth factor 23 (FGF23) plays an important role in independent pathophysiological mechanisms among cardiovascular disease risk factors in chronic kidney disease [8–10]. Epidemiologic studies suggest that elevation of FGF23 is strongly associated with left ventricular hypertrophy (LVH) and that high FGF23 is an independent risk factor for congestive heart failure (CHF) and mortality [11, 12].

FGF23 is a phosphatonin that regulates phosphate levels in the intestine, bone and kidneys. Previous studies have shown that FGF23 elevation is associated with LVH in patients with CRF who do not undergo dialysis. Increased FGF23 concentrations in hemodialysis patients

have been linked to increased mortality in the first year of dialysis treatment [13]. Studies in the general population have shown that brain natriuretic peptide (BNP) is a more potent marker of LVH and systolic dysfunction than other peptides [14]. Mallamaci and colleagues have shown that cardiac natriuretic peptides (especially BNP) are sensitive molecules in the diagnosis of LVH and systolic dysfunction in dialysis patients [15].

MATERIALS & METHODS

After obtaining ethical committee approval and consent from the parents, we included 20 patients, who underwent peritoneal dialysis in the peritoneal dialysis unit of a medical university, Turkey. Exclusion criteria were as follows: patients who were younger than 18 years of age, who had cancer, who had experienced physical trauma within the last month, who had undergone a coronary bypass, who had a surgical operation or burn history who exhibited the presence of acute infection, whose daily urine output was <300mL or who had a history of symptomatic or decompensated liver disease, myocardial infarction, peripheral artery disease or cerebrovascular disease.

Patients were evaluated for age, gender, height, weight, body mass index (BMI), daily urine output, CRF duration, etiology, glomerular filtration rate (GFR) values, hemogram, serum creatinine, blood urea nitrogen (BUN), calcium, phosphorus, albumin and parathormone levels. This assessment was made at the beginning and end of the study i.e. at an interval of three-months. Blood samples were taken for FGF23, NGAL and N-terminal pro-brain natriuretic peptide (NT-proBNP) in patients two times in the beginning and the third month of follow-up. Transthoracic echocardiography was performed two times by the same cardiologist at the beginning and end of the follow-up period.

Twenty healthy individuals with no known active diseases were selected as a control group in the same age range as the patients and in them, FGF23, NGAL, NT-proBNP and fetuin levels were studied. Heparinized plasma was used in the study, and the collected blood was stored at -70 °C before the study. At the beginning of the study, the samples were allowed to stand at room temperature for dissolution. Lipocalin-2/NGAL was studied with the ELISA method on a Brio-brand Seac SRL-model device (BioVendor GmbH, D-69120 Heidelberg, Germany) with the BioVendor-brand kit (Brio, Seac SRL, Radim Company, Calenzano, Firenze, Italy). Before starting to work, the lyophilized stock in the kit was dissolved in a standard dilution buffer at a concentration of 10 ng/mL. Serial dilutions were performed at concentrations of 5 ng/mL, 2.5 ng/mL, 1.25 ng/mL, 0.6 ng/mL and 0.3 ng/mL.

Plasma samples stored at -70 °C were thawed at room temperature and diluted 30-fold with the dilution buffer. Diluted 100 µL was taken from the samples and pipetted into 96 plate-coated wells with a lipocalin-2/NGAL antibody. Each well was washed three times with 350 µL of wash solution after incubation for one hour at the plate room temperature. After washing, 100 µL of Streptavidin-HRP solution was pipetted into each of the plates, and then the plate surface was sealed and incubated for 30 minutes at room temperature. In addition, 100 µL of stabilized chromogen (substrate) was added to the plate following the washing step. The plate incubation was carried out in the dark at room temperature for 10 minutes. A stop solution containing 100 µL H2SO4 was added to the plates, and the reaction was stopped. At the end of the study, the optical density (OD) in the plates was evaluated in an ELISA reader (ELX800, BIO-TEK Instruments) at a wavelength of 450 nm and the results were calculated in ng/mL.

FGF23 assay was performed by ELISA method (Brio, Seac SRL, Radim Company, Calenzano, Firenze, Italy). A Millipore kit was used (Millipore Corporation, Billerica, MA, USA), working on a Brio Seac SRL-model device. Before starting to work, the lyophilized stock in the kit was dissolved in 0.5 mL of deionized water at a concentration of 7200 pg/mL. Standards were prepared at 2400 pg/mL, 800 pg/mL, 266 pg/mL, 88pg/mL, 29 pg/mL and 9 pg/mL concentrations by serial dilution with an assay running buffer. After adjusting the wells for blank, standard, control and samples, 50 µL of the matrix solution was pipetted into these wells. Again, blank and sample wells were pipetted into 50 µL wells from the assay running buffer. Samples were taken 50 µL from the standard and controls and pipetted into wells that were previously detected. After incubation for two hours at plate temperature, each well was washed three times with 300 µL of wash solution. Then 100 µL of detection antibody solution was added to all wells, and the surface of the plate was closed. After incubation for one hour at the plate room temperature, each well was washed three times with 300 µL of wash solution. Subsequently, 100 µL of enzyme solution was pipetted into each cassette, and the plate surface was sealed and incubated for 30 minutes at room temperature. Each well was washed three times with 300 µL of wash solution, and 100 µL of stabilized chromogen (substrate) was added to the plate. After incubation of the plate in the dark at room temperature for 20 minutes, stop solution containing 100 µL H2SO4 was added to the plate and the reaction was stopped. At the end of the study, the OD in the plates was evaluated in an ELISA reader (ELX800, BIO-TEK Instruments) at a wavelength of 450 nm. Results are given in pg/mL.

The NT-proBNP was run on a Siemens-branded Immulite 2000 device using the chemiluminescence method (Siemens Healthcare Diagnostics Products Ltd.,

Llanberis, Gwynedd LL55 4 EL, United Kingdom). The results of the work performed on the fully automated device that has been checked and calibrated are given in pg/mL.

Statistical analysis was as follows: the square sample t-test was used for comparison of normal-range measures. The Mann-Whitney U-test was used for comparison of non-normalized measurements, and $p < 0.05$ was considered statistically significant. Wilcoxon signed rank tests and paired t-tests were used according to their distributions for comparison of recurrent measurements. Spearman correlation analysis was performed. Multivariate regression analysis was used to find determinants of serum NGAL levels.

RESULTS

There were 12 males and 8 females in both the group i.e. patient and control group. No significant difference was found between the patient and control groups in terms of gender and age ($p > 0.05$). A statistically significant difference was found between GFR, modification of diet in renal disease (MDRD), BUN, creatinine, proBNP, NGAL and FGF23 levels between the patient and control groups ($p < 0.05$). The GFR and MDRD were higher in the control group while BUN, creatinine, proBNP, NGAL and FGF23 levels were higher in the patient group (Table 1).

In the patient group, there was no significant difference in terms of sex, age, GFR, MDRD, calcium, phosphorus or BMI between patients with proBNP >1000 and proBNP <1000 ($p > 0.05$). Among the two groups, albumin was significantly different between the first and second evaluation of proBNP and these two evaluations ($p < 0.05$). Albumin was lower in the loaded group with proBNP > 1000 pg/mL (Table 2).

Between the two groups, there was no significant difference in BUN, creatinine, parathormone, proBNP, NGAL, FGF23, LVEDD measurement, left atrium diameter measurement, aortic root measurement and residual urine volume, at first and second evaluation ($p > 0.05$). There was no statistically significant difference between two groups of first and second NGAL, FGF23 and MDRD measurements ($p > 0.05$).

In the evaluation of patients with a proBNP level <1000 pg/mL, BUN, creatinine, parathormone, MDRD, proBNP, NGAL, FGF23, LVEDD and left atrial diameter were found to be significant in terms of two values ($p > 0.05$). The difference between the two measurements of the aortic root diameter and residual urine in initial evaluation of patients with a proBNP <1000 pg/mL was statistically significant ($p < 0.05$). In patients with a proBNP <1000 pg/mL, aortic root diameter was higher in the first measurement, while residual urine was higher in the second measurement (Table 3).

Table 1: Comparison of initial assessment of patient and control group

Parameters Tested	Patient group, n: 20 (Mean ± SD)	Control group, n: 20 (Mean ± SD)	p-value
Gender (female) n (%)	9 (45%)	8 (40%)	0.500
Age (year)	44.8 ± 15.9	35.3 ± 4.8	0.063
GFR (mL/min.)	12.50 ± 5.5	130.6 ± 31.5	0.000
MDRD (mL/min.)	8.5 ± 4.1	97.8 ± 30.0	0.000
BUN (mg/dL)	55.4 ± 20.9	12.0 ± 2.5	0.000
Creatinine (mg/dL)	7.5 ± 2.8	0.8 ± 0.2	0.000
proBNP (pg/mL)	6839.4 ± 10168.4	46.7 ± 23.0	0.000
NGAL (ng/mL)	186.7 ± 51.2	83.1 ± 29.1	0.000
FGF23 (pg/mL)	455.9 ± 621.7	20.8 ± 16.7	0.000

GFR: glomerular filtration rate; MDRD: Modification of Diet in Renal Disease; BUN: blood urea nitrogen; proBNP: N-terminal pro-brain natriuretic peptide; NGAL: neutrophil gelatinase-associated lipocalin; FGF23: fibroblast growth factor 23.

The difference between the two measurements of BUN, proBNP and aortic root diameter in the initial evaluations of patients with proBNP >1000 pg/mL was statistically significant ($p < 0.05$). In all three parameters, the first measurement was higher. The difference between the two measurements of creatinine, parathormone, MDRD, NGAL, FGF23, LVEDD, left atrium diameter and residual urine was not statistically significant in the initial evaluations of patients with proBNP >1000 pg/mL ($p > 0.05$).

Table-2: Table 2: Comparison of patient group with proBNP <1000 pg/mL and >1000 pg/mL

Patient group	Pro-BNP >1000 pg/mL n = 10, (Mean±SD)	Pro-BNP <1000 pg/mL n = 10, (Mean±SD)	p-value
Gender (female), n (%)	6 (60%)	5 (50%)	0.684
Age (year)	44.7 ± 19.8	44.8 ± 11.7	0.684
GFR (mL/min.)	12.7 ± 4.8	12.3 ± 6.4	0.739
MDRD1 (mL/min.)	9.5 ± 4.2	7.5 ± 3.8	0.76
Albumin (gr/dL)	3.1 ± 0.7	3.7 ± 0.3	0.023
Calcium (mg/dL)	8.6 ± 0.6	9.0 ± 0.8	0.481
Phosphorus (mg/dL)	5.5 ± 1.7	5.0 ± 1.6	0.529
BMI (kg/m^2)	22.9 ± 3.6	24.7 ± 5.6	0.393
BUN1 (mg/dL)	52,9 ± 16,5	57,9 ± 25,3	0.971
BUN2 (mg/dL)	42,3 ± 17,1	47,1 ± 8, 8	0.247
Creatinine1 (mg/dL)	6,9 ± 2,8	8,0 ± 3,0	0.247
Creatinine2 (mg/dL)	7,2 ± 2,9	7,1 ± 2,7	0.971
PTH1 (pg/mL)	363,8 ± 278,1	374,8 ± 175,5	0.529
PTH2 (pg/mL)	342,1 ± 334,6	266,8 ± 141,7	0.540
proBNP1 (pg/mL)	13323,3 ± 11235,9	456,1 ± 266,7	0.000
proBNP2 (pg/mL)	9667,3 ± 9483,2	1257,0 ± 2418,0	0.000
NGAL1 (ng/mL)	178,9 ± 47,0	194,4 ± 56,5	0.481
NGAL2 (ng/mL)	191,5 ± 53,7	228,3 ± 73,5	0.190
FGF23.1 (pg/mL)	537,5 ± 733,9	374,3 ± 512,5	0.853
FGF23.2 (pg/mL)	747,5 ± 1099,5	498,3 ± 821,9	0.739
LVEDD 1 (cm)	4, 8 ± 0,7	4,4 ± 0,3	0.408
LVEDD 2 (cm)	4.7 ± 0.7	4.6 ± 0.4	0.696
Left atr d 1 (cm)	3.5 ± 0.7	3.2 ± 0.3	0.299
Left atr d 2 (cm)	3.5 ± 0.7	3.5 ± 0.3	0.965
Aortic root 1 (cm)	2.6 ± 0.4	2.9 ± 0.6	0.351
Aortic root 2 (cm)	2.5 ± 0.4	2.5 ± 0.3	0.965
Residual urine1 (mL/day)	805.0 ± 226.6	870.0 ± 249.7	0.481
Residual urine 2 (mL/day)	835.0 ± 266.7	960,0 ± 275,7	0.353
Δ proBNP (pg/mL)	6441.0 ± 8528.2	-800.9 ± 2245.4	0.001

Patient group	Pro-BNP >1000 pg/mL n = 10, (Mean±SD)	Pro-BNP <1000 pg/mL n = 10, (Mean±SD)	p-value
Gender (female), n (%)	6 (60%)	5 (50%)	0.684
Age (year)	44.7 ± 19.8	44.8 ± 11.7	0.684
GFR (mL/min.)	12.7 ± 4.8	12.3 ± 6.4	0.739
MDRD1 (mL/min.)	9.5 ± 4.2	7.5 ± 3.8	0.76
Δ NGAL (ng/mL)	-12.6 ± 51.8	-33.8 ± 88.1	0.739
Δ FGF23 (pg/mL)	-20.9 ± 60.5	-4.8 ± 15.9	0.912
Δ MDRD (mL/min.)	0.4 ± 1.4	-1.2 ± 3.8	0.315

GFR: glomerular filtration rate; MDRD: Modification of Diet in Renal Disease; BMI: body mass index; BUN: blood urea nitrogen; PTH: parathormone; proBNP: N-terminal pro-brain natriuretic peptide; NGAL: neutrophil gelatinase-associated lipocalin; FGF23: fibroblast growth factor 23; LVEDD: left ventricular end-diastolic diameter; Left atr d: left atrium diameter; Aortic root: aortic root width; Residual urine: residual urine volume. The number 1 at the end of the units is added to indicate the first evaluation, and the second number is the second evaluation. Δ proBNP is the difference between the first and second evaluation of proBNP; Δ NGAL is the difference between the first and second evaluation of NGAL; Δ FGF23 is the difference between the first and second evaluation of FGF23; Δ MDRD is the difference between the first and second evaluation of MDRD.Accuracy comparisons within groups were demonstrated in table 3 and table 4. The highest marginal gap value was seen in proximal view of the group H (123.6±6.1 µm), and the highest internal gap value was detected in axial view of the same group (233.9±12.6 µm) (p<0.001).

Table-3: Analysis of two separate measurements of first assessments of patients with proBNP < 1000 pg/mL

Parameters	Pro-BNP <1000 pg/mL n = 10, (Mean±SD) First Measurement	Pro-BNP <1000 pg/mL n = 10, (Mean±SD) Second Measurement	p-value
BUN (mg/dL)	57.9 ± 25.3	47.1 ± 8.8	0.167
Creatinine (mg/dL)	8.0 ± 3.0	7.1 ± 2.7	0.284
PTH (pg/mL)	374.8 ± 175.5	266.8 ± 141.8	0.047
MDRD (mL/min.)	7.5 ± 3.8	8.7 ± 4.5	0.424
proBNP (pg/mL)	456.1 ± 266.7	1257.0 ± 2418.0	0.093
NGAL (ng/mL)	194.4 ± 56.5	228.3 ± 73.5	0.646
FGF23 (pg/mL)	374.3 ± 512.5	498.3 ± 821.9	0.114
LVEDD (cm)	4.4 ± 0.3	4.6 ± 0.4	0.705
Left atr d (cm)	3.2 ± 0.3	3.5 ± 0.3	0.136
Aortic root (cm)	2.9 ± 0.6	2.5 ± 0.3	0.046
Residual urine (mL/day)	870.0 ± 249.7	960.0 ± 275.7	0.041

BUN: blood urea nitrogen; PTH: parathormone; MDRD: Modification of Diet in Renal Disease; proBNP: N-terminal pro-brain natriuretic peptide; NGAL: neutrophil gelatinase-associated lipocalin; FGF23: fibroblast growth factor 23; LVEDD: left ventricle end-diastolic diameter; Left atr d: left atrium diameter; Aortic root: aortic root width; Residual urine: residual urine volume.

On multivariate analysis, performed with the aim of determining serum NGAL level determinants, the albumin effect was found to be statistically significant ($p < 0.05$). However, the difference between the first group of patients, age and proBNP was not statistically significant ($p > 0.05$) (Table 5).

Table-4: Comparison of internal gap values within groups.

Parameters	Pro-BNP >1000 pg/mL n = 10, (Mean±SD) First Measurement	Pro-BNP >1000 pg/mL n = 10, (Mean±SD) Second Measurement	p-value
BUN (mg/dL)	52.9 ± 16.5	42.3 ± 17.1	0.009
Creatinine (mg/dL)	6.9 ± 2.8	7.2 ± 2.9	0.540
PTH (pg/mL)	363.8 ± 278.1	342.1 ± 334.6	0.739
MDRD (mL/min.)	9.5 ± 4.2	9.1 ± 4.3	0.395
proBNP (pg/mL)	13323.3 ± 11235.9	9667.3 ± 9483.2	0.035
NGAL (ng/mL)	178.9 ± 47.0	191.5 ± 53.7	0.333
FGF23 (pg/mL)	537.5 ± 733.9	747.5 ± 1099.5	0.203
LVEDD (cm)	4.8 ± 0.7	4.7 ± 0.7	0.414
Left atr d (cm)	3.5 ± 0.7	3.5 ± 0.7	0.144
Aortic root (cm)	2.6 ± 0.4	2.5 ± 0.4	0.002
Residual urine(mL/day)	805.0 ± 0.4	835.0 ± 266.7	0.334

BUN: blood urea nitrogen; PTH: parathormone; MDRD: Modification of Diet in Renal Disease; proBNP: N-terminal pro-brain natriuretic peptide; NGAL: neutrophil gelatinase-associated lipocalin; FGF23: fibroblast growth factor 23; LVEDD: left ventricle end-diastolic diameter; Left atr d: left atrium diameter; Aortic root: aortic root width; Residual urine: residual urine volume.

Table 5: Factors affecting baseline serum NGAL level and of serum NGAL measured at the end of the 3rd month on multivariate analysis

Parameter	Variables R^2: 0.245		
Depended variables	Independent variables	β	p
NGAL1	Albumin	0.495	0.026
	Patient group	-0.143	0.565
	Age	0.097	0.670
	NT-proBNP1	0.161	0.502
NGAL2	Albumin	0.525	0.017
	Patient group	0.016	0.948
	Age	0.194	0.381
	Δ proBNP	0.203	0.371

NGAL2: second evaluation of patients with neutrophil gelatinase-associated lipocalin; NT-proBNP1: first measurement of proBNP, Δ proBNP: difference between the first and second evaluations of proBNP.

The effect of albumin on the second measurement of NGAL was statistically significant ($p < 0.05$). However, the difference between the first group of patients, age and proBNP was not statistically significant ($p > 0.05$).

DISCUSSION

In our study, the mean serum NT-proBNP level was 6839.4±10168.4 pg/mL in the patient group, and 46.7±23.0 pg/mL in control group. The mean serum NGAL level was 186.7±51.2 ng/mL in patients, while the control group had a mean of 83.1±29.1 ng/mL. The mean serum FGF23 level was 455±621.7 pg/mL in patients and 20.8±16.7 in controls; serum NT-proBNP and NGAL levels of patients were significantly higher than in the control group ($p < 0.05$). In patients with renal insufficiency, an increase in serum NT-proBNP and NGAL levels may be expected due to decreased clearance.

According to established medical guidelines [16], the cut-off value to diagnose heart failure is 100 pg/mL (28.9 pmol/L) for BNP and 300 pg/mL (35.4 pmol/L) for NT-proBNP, in patients with normal renal function. We preferred to use different cut-off values since there was a decrease in BNP or NT-proBNP clearances in patients with impaired renal function.

Naganuma and colleagues reported an increased risk of cardiac death when plasma values were greater than 700 pg/mL (200 pmol/L) in hemodialysis patients [17]. Wang et al found [18] that cardiovascular mortality and morbidity rates in peritoneal dialysis patients were the lowest when NT-pro-BNP levels were below 1928 pg/mL (227.5 pmol/L), and the highest when they were above 17534 pg/mL (2069 pmol/L). In our study, we examined patients with NT-proBNP levels above and below 1000 pg/mL. Mean serum NT-proBNP levels in the hypervolemic group, with serum NT-proBNP levels above 1000 pg/mL, were found (13323.3 ± 11235.9 pg/mL). The mean serum NT-proBNP levels in the group with NT-proBNP levels below 1000 pg/mL were 456.1 ± 266.7 pg/mL ($p < 0.05$)

Damman et al. reported impairment in renal function and an increase in NGAL levels due to venous congestion in patients with right-sided heart failure. These investigators reported that NGAL in cardiac insufficiency is a good indicator of impairment of renal function [19]. Daniels et al found a relationship between NGAL and proBNP in a study of 1,393 Rancho Bernardo Study participants for an average of 11 years, and they found that NGAL is a strong indicator of death from cardiovascular disease and death due to all other causes [20]. They reported that this was independent of the change in GFR rate of the patients. NGAL could be used as an additional factor for other determinants such as NT-proBNP and C reactive protein for cardiovascular mortality. These investigators report that NGAL has a poor association with traditional cardiovascular risk factors such as age, systolic blood pressure, hypertension and diabetes.

In this study of patients with residual renal function, we compared patients with severe hypervolemic peritoneal dialysis who had similar estimated GFR calculated by the Cockroft-Gault and Modification of Diet in Renal Disease study formulas and patients with peritoneal dialysis patients whose volume control was better with serum NGAL levels. There was no correlation between NGAL levels and LVEDD, left atrium diameter, aortic root width or residual urine volume. Similarly, Koca and colleagues found no association between NGAL levels and heart failure in their studies of hypertensive kidney function in normal patients with right-left heart failure [21].

In our study, we evaluated the serum NT-proBNP and NGAL levels after three months to assess changes in volume status of patients and their effect on serum NT-proBNP and NGAL levels. Serum NT-proBNP levels in patients with NT-proBNP levels >1000 pg/mL (hypervolemic group) and those with NT-proBNP levels <000 pg/mL (better volume control) at the first measurement compared to the initial measurements (13323.3±11235.9pg/ml) were significantly decreased ($p <0.05$). There was no significant change in NGAL levels. We found no significant change in serum NT-proBNP and NGAL levels in patients with better control of volume with NT-proBNP levels <1000 pg/mL. There was no difference in the predicted GFR calculated by the Cockroft-Gault and MDRD study formulas between the two groups after three months of follow-up.

In the multivariate regression analysis to determine the determinants of serum initial measurement of NGAL, we identified albumin as an independent determinant of NGAL when it was taken as a model group 1 (hypervolemic group), age, serum albumin and NT-proBNP. In the multivariate regression analysis to determine the determinants of serum NGAL second measurement, we also found albumin as an independent predictor of NGAL when the change in age, serum albumin and serum NT-proBNP levels were taken as group 1 (hypervolemic group). We saw that the other factors did not have an effect.

The existence of NGAL on atherosclerotic plaques has been detected in studies. Vascular cells are most likely induced by the expression of NGAL during atherogenesis. However, the induction mechanism of NGAL in vascular cells is still unknown [22]. High FGF23 levels have been found to be associated with bone mineralization [23], pre dialysis progression in patients with CRF [24], LVH [25, 26] and mortality in hemodialysis patients [27, 28]. In our study, we could not identify a relationship between NGAL and FGF23 levels.

As a result, serum NT-proBNP and NGAL levels were significantly higher in the patient group than in the healthy control group. However, we could not detect a correlation between the levels of serum NT-proBNP and NGAL, which were evaluated as a sign of body volume status. Although our study is limited by the fact that it was performed on very few patients, it is important to note that other factors may have effects on serum NGAL. These include extracellular serum albumin in peritoneal dialysis patients and variables that we cannot fit into the multivariate analysis model.

CONCLUSION

The relation between serum NT-proBNP and NGAL levels and there are several other factors that may have effects on serum NGAL. This fact indicates the need for a more comprehensive study of hypervolemia and the reduction of NGAL and residual renal function, which is often encountered in patients with renal insufficiency.

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