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Fibroblast Growth Factor-23, Matrix Gla Protein and the Incident of Cardiovascular Events in Chronic Haemodialysis Patients with No Pre-existing Cardiovascular Disease: a One Centre Cohort Study

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Summary

Background. Dialysis patients are known to have an increased risk of total and cardiovascular mortality. Previously we described that vascular calcification (VC) is an independent predictor of novel non-fatal cardiovascular events in haemodialysis (HD) patients. Fibroblast growth factor-23 (FGF 23) and Matrix Gla protein (MGP) are known to be important contributors for VC and mortality in HD patients. There have been studies analysing the associations between FGF 23, MGP and cardiovascular diseases, but not in the special cohort of maintenance HD patients.

Objective. To analyse the possible associations between biomarkers (FGF 23, MGP) and the incidence of cardiovascular diseases in HD patients.

Methods. This observational prospective single-centre study included 75 prevalent patients on maintenance HD. After evaluating VC, heart ultrasound, laboratory tests, levels of MGP and FGF 23, patients were observed for the incident of non-fatal cardiovascular events. A detailed analysis was performed in groups with (CVD group) and without (no CVD group) pre-existing cardiovascular diseases.

Results. Neither biomarkers nor heart ultrasound measures differed significantly in patients with and without new cardiovascular events in the CVD group. In the no CVD group univariate Cox regression analysis showed that the relative risk of novel cardiovascular event was 5.5 higher in patients with a FGF 23 level lower than 41 ng/L ($p=0.008$, 95% CI 1.6–19.7) and 5.9 higher in patients with a MGP level lower than 1.64 ng/mL ($p=0.006$, 95% CI 1.7–21).

Conclusions. We report a novel association of low FGF 23 and MGP levels and the incident of cardiovascular events in HD patients without pre-existing cardiovascular diseases.

Keywords: dialysis, biomarkers, cardiovascular diseases.

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INTRODUCTION

Patients on renal replacement therapy are known to have increased total and cardiovascular mortality [1–3]. Cardiovascular mortality in haemodialysis (HD) patients is higher when compared to the general population [4]. This increased cardiovascular risk results from the interplay of traditional (e.g. hypertension, dyslipidaemia, diabetes) and non-traditional cardiovascular risk factors. Calcification in the media layer of the vessel wall is specific for HD patients and is one of the central contributors to cardiovascular disease and mortality [5, 6]. Previously we confirmed that VC assessed by simple radiological method was an independent predictor of novel non-fatal cardiovascular events in HD patients [7]. VC is not considered as a passive but rather as an actively regulated process dependent on calcification promoters and inhibitors. Clinical studies indicate that fibroblast growth factor 23 (FGF 23) and Matrix Gla protein (MGP) may play an important role in this process [8–10].

We, therefore, analysed a cohort of HD patients testing the hypothesis that not only VC but also FGF 23 and MGP levels may be important in predicting the incident of cardiovascular events.

METHODS

This observational prospective single-centre study included prevalent patients (n=75) on maintenance HD in the dialysis unit of Hospital of Lithuanian University of Health Sciences Kaunas Clinics.

The study was approved by Kaunas Regional Biomedical Research Ethics Committee (protocol number BE-2-9 on 2014-04-01). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent was obtained from all individual participants included in the study.

Demographical, clinical data, history of pre-existing cardiovascular diseases, data of heart ultrasound were collected from medical records of each patient. Measurement of VC was counted as a beginning of the follow-up. After measurement of VC, HD patients were observed for incident non-

fatal cardiovascular events (primary end-point of the study). Patients were followed from the baseline examination until the first occurrence of a cardiovascular event or until the end of May 2017. Mean follow up 979 days (23–1560). The detailed analysis was performed after dividing patients into groups according to the history of the cardiovascular diseases. Patients with cardiovascular disease diagnosed before the study start were assigned to the cardiovascular disease (CVD) group (29 patients). The no CVD group consisted of patients without any known CVD before the study start (46 patients).

Cardiovascular events included coronary, cerebrovascular and peripheral vascular morbidity as it is described previously [7]. If any of these conditions have been found, the patient was considered as having cardiovascular disease.

Patients with self-reported or physician-reported diabetes mellitus or currently using hypoglycaemic medication were categorised as diabetic. The patient was determined as hypertensive if the physician reported hypertension and/or antihypertensive medicine was prescribed. HD duration, as well as age, were evaluated on the day of simple vascular calcification score (SVCS) assessment.

SVCS was evaluated in all patients as it is described by Adragao et al. [11] with SVCS 3 considered as a cut-off value.

Blood samples for the measurement of biomarkers were collected at the same month as SVCS was evaluated. The whole blood was collected into plane tubes and was allowed to clot by leaving it at room temperature for 20 minutes. Serum was separated by centrifugation at 2500×g for 15 minutes. All serum samples were stored at -80 °C until analysis. Human FGF 23 ELISA Kit (Sunlong Biotech, Zhejiang China) was used for analysis

of FGF 23. Human MGP ELISA Kit (Sunlong Biotech, Zhejiang China) was used for analysis of MGP. For the detailed analysis patients were stratified according to FGF 23 and MGP median levels (41 vs >41 ng/L and 1.64 vs >1.64 ng/mL respectively).

Statistical analysis

Variables were expressed as frequencies and percentages for discrete factors. For continuous factors mean values ± standard deviation and median with interquartile range were used where appropriate. A statistical comparison was performed using the two-tailed chi-square test for categorical variables, two-tailed Student's T-test or Mann-Whitney test for continuous variables. Kaplan–Meier and Cox's regression were used to evaluate time to an incident cardiovascular event. For all comparisons a p value < 0.05 was considered statistically significant. Statistical analysis was performed using SPSS 24 software package.

RESULTS

Results of 75 patients were analysed: 40 (53%) men and 35 (47%) woman. The mean age of patients was 60±15.8 year, the median of HD vintage was 19.5 month (1–82). 15 (20%) patients were diabetic, 69 (82%) had hypertension. Clinically relevant VC with SVCS 3 was diagnosed in 44 (59%) patients. On the heart ultrasound the mean myocardial mass index (MMI) was 122.6±30.6 gm/m², mean ejection fraction 51.2±7.6; in 61 (81%) patients ejection fraction was higher than 50%. Median level of FGF 23 was 41.01 ng/L (0.96–199.79 ng/L), median level of MGP was 1.64 ng/mL (0.41–6.36 ng/mL).

During the follow-up period new CV events were diagnosed in 22 pa-

Table 1. Factors associated with incident cardiovascular events in haemodialysis patients with no pre-existing cardiovascular events

	Incident cardiovascular event group (n=15)	No cardiovascular event group (n=31)	p
Age (years)	63.4±15.6	55.9±15.8	N.S.
FGF 23 ng/L	20.2 [0.96–60]	53.87 [1–199]	0.004
FGF 23 >41 ng/L	3 (20%)	22 (71%)	0.001
MGP ng/mL	1.32 [0.65–2.58]	1.88 [0.51–5.96]	0.002
MGP >1.6 ng/mL	3 (20%)	22 (71%)	0.001
MMI gm/m ²	137 [97–216]	104 [28–183]	0.004
SVCS	4.8±2.6	2.58±2.6	0.011

Results in mean values±SD and median [IQR], were appropriate, or n (%); SVCS – simple vascular calcification score; N.S. – not significant.

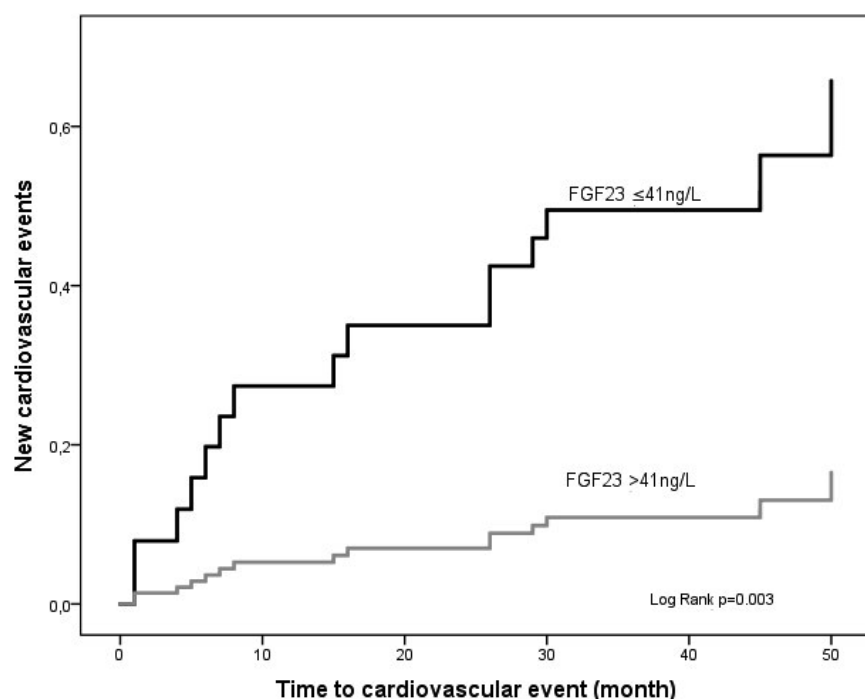


Fig. 1. Incident cardiovascular events in haemodialysis patients with no pre-existing cardiovascular diseases in groups of patients with FGF 23 ≤ 41 ng/L vs. >41 ng/L

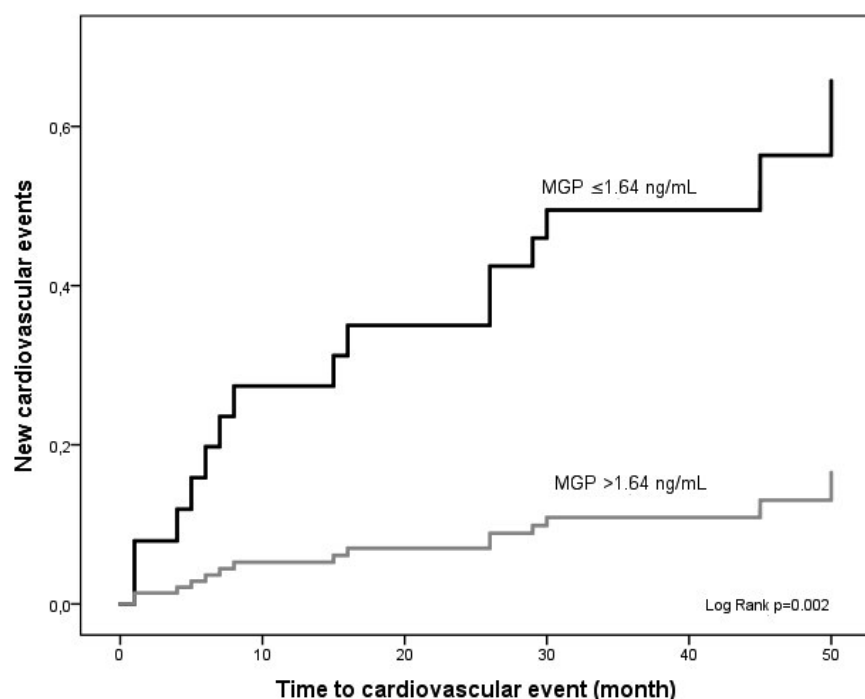


Fig. 2. Incident cardiovascular events in haemodialysis patients with no pre-existing cardiovascular diseases in groups of patients with MGP ≤ 1.64 ng/mL vs. >1.64 ng/mL

tients (29%). No relation between FGF 23, MGP levels and new cardiovascular events was found in the whole study population. However, biomarkers became important when patients were divided into groups according to the history of CVD (CVD group vs no CVD group). For detailed investigation these groups were analysed separately. No differences between

these groups were found in age, dialysis vintage, laboratory test results, FGF 23, MGP levels and MMI. The ejection fraction was higher in the no CVD group ($53 \pm 6\%$) as compared to the CVD group ($48.6 \pm 9\%$), $p=0.03$.

During the follow-up 7 (24%) and 15 (32%) new CV events were diagnosed in the CVD group and the no CVD group accordingly, $p=0.4$.

Afterwards, groups were analysed separately and we found that neither biomarkers nor ultrasound measures differed significantly between patients with and without new CV events in CVD group. Factors associated with new cardiovascular events in the no CVD group presented in Table 1.

In the group of patients without pre-existing CVD, univariate Cox regression analysis revealed that relative risk of incident cardiovascular event was 5.5 higher in patients with FGF 23 levels lower than 41 ng/L ($p=0.008$, 95% CI 1.6–19.7) (Fig. 1) and 5.9 higher in patients with MGP levels lower than 1.64 ng/mL ($p=0.006$, 95% CI 1.7–21) (Fig. 2). No significant differences were observed comparing laboratory tests (haemoglobin, albumin, C reactive protein, total calcium, phosphate, total cholesterol), dialysis vintage, ejection fraction between the subgroups with and without new CV events in the no CVD group of patients.

Factors important for the risk of incident cardiovascular events in the no CVD group in univariate analysis were included in multivariate Cox regression analysis. Because of a strong correlation between FGF 23 and MGP levels (Spearman's coefficient 0.73, $p<0.001$) (Fig. 3) these two factors could not be included in the same model and were analysed separately (Table 2 and 3). As it is demonstrated in Tables 2 and 3, the significance of MGP and FGF 23 was not suppressed by SVCS and MMI so they could be considered as independent factors associated with novel CV event in HD patients with no pre-existing CVD.

DISCUSSION

In the current study, we, for the first time, reported an association between low FGF 23 and MGP levels and the occurrence of new cardiovascular events in maintenance HD patients without pre-existing cardiovascular diseases.

During the past decade, reports of clinical studies have described the association between FGF 23 and cardiovascular risk, left ventricular hypertrophy (LVH) and VC [10, 12, 13]. Impaired calcium-phosphate metabolism was shown to be associated with chronic heart failure [14].

In dialysis patients, higher FGF 23 levels have been linked to increased mortality rates and VC [15]. In two cohort studies incident [16] and prevalent [5] haemodialysis patients with

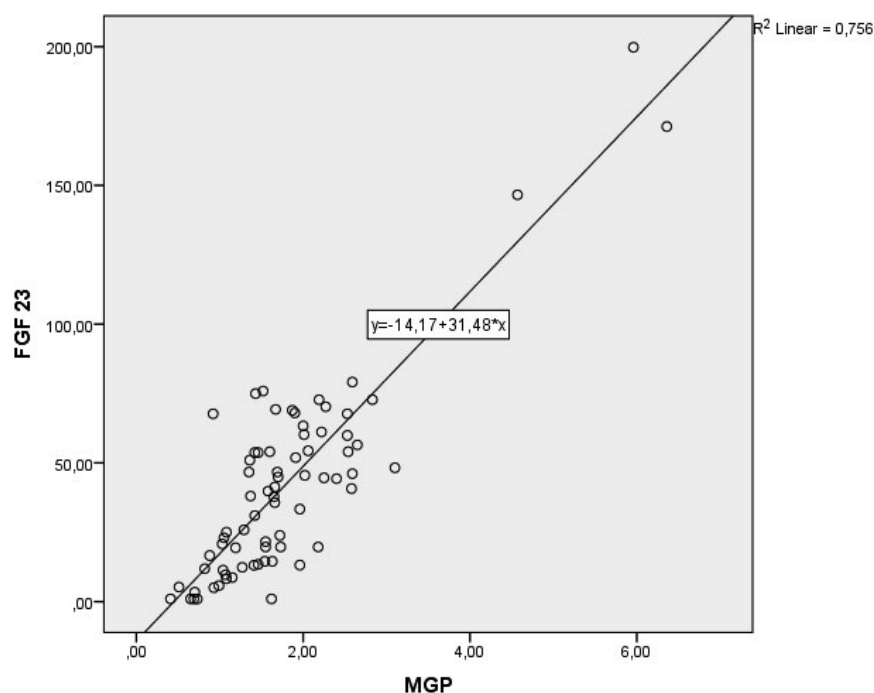


Fig. 3. Correlation between FGF 23 and MGP in haemodialysis patients

Table 2. Factors, associated with incident cardiovascular events in haemodialysis patients with no pre-existing cardiovascular diseases (Cox regression for FGF 23)

Factors	Relative risk	p	95% CI for relative risk
FGF 23 41 ng/L	11.7	0.001	2.9–48.2
SVCS	1.3	0.01	1.1–1.7
MMI	1.05	<0.001	1.02–1.07

FGF 23 – fibroblast growth factor-23; SVCS – simple vascular calcification score; MMI – myocardial mass index.

Table 3. Factors, associated with incident cardiovascular events in haemodialysis patients with no pre-existing cardiovascular diseases (Cox regression for MGP)

Factors	Relative risk	p	95% CI for relative risk
MGP 1.64 ng/mL	3.9	0.04	1.1–14.7
SVCS	1.3	0.02	1.04–1.6
MMI	1.03	0.004	1.01–1.05

MGP – matrix Gla protein; SVCS – simple vascular calcification score; MMI – myocardial mass index.

highest FGF 23 serum levels had the highest mortality during follow-up even after adjustment for serum phosphate levels.

The primary end point in most of the studies evaluating the impact of FGF 23 levels on outcomes of haemodialysis patients was mortality. There have been a number of studies analysing associations between FGF 23 levels and cardiovascular risk, but most of them evaluated patients with CKD and peritoneal dialysis patients, but not patients on maintenance haemodialysis.

In patients with chronic kidney disease, higher plasma FGF 23 con-

centrations were strongly associated with all-cause mortality, cardiovascular events and progression to dialysis, independent of established risk factors such as age, hypertension, diabetes, smoking, kidney function and abnormalities of mineral metabolism [17]. The Heart and Soul Study also confirmed that in patients with stable coronary artery disease and impaired kidney function, higher FGF 23 levels and lower ucMGP levels are associated with mortality and cardiovascular events independently of traditional risk factors, kidney function, and C-reactive protein levels [18]. Bech and co-authors found that

FGF 23 itself, but not its renal tissue resistance as reflected by FePi, is an important risk factor for clinical events such as death, renal failure and cardiovascular events in subjects with CKD stage 3–4 [19]. A single centre retrospective study on peritoneal dialysis patients performed by Kim and co-authors revealed that after adjustment for age, DM, pre-existing coronary artery disease, cerebrovascular disease, congestive heart failure and left ventricular mass index the higher FGF 23 group exhibited significantly higher risk of cardiovascular events [20].

However, all these studies were performed on pre-dialysis patients, with different stages of CKD or peritoneal dialysis patients, but not patients on maintenance haemodialysis. In our study we did not find the associations of higher FGF 23 levels and new cardiovascular events in haemodialysis patients in agreement with the study by Nakano and al., which revealed that intact FGF 23 levels in pre-dialysis CKD patients predicted incident cardiovascular events requiring hospitalization before starting dialysis, but not during the entire follow-up period, including post dialysis initiation period [21]. We found that lower FGF 23 levels were associated with new cardiovascular events in patients without pre-existing cardiovascular diseases which supports the data published by Moldovan and co-authors who reported an association between low circulating FGF 23 and the presence of cardiovascular diseases in chronic HD patients. In their study, the patients with CVD were older and had more extended VC. They were significantly more affected by protein malnutrition and anaemia. Advanced age and low albumin were recognised as additional risk factors for CVD [22]. We could not confirm the influence of malnutrition on occurrence of new cardiovascular events, but we found that SVCS 3 was associated with a higher relative risk of novel non-fatal cardiovascular events in haemodialysis patients as it was published previously [7].

LVH is an important factor for CVD in individuals with CKD. Elevated levels of FGF 23 have been linked to greater risks of LVH and mortality in patients with CKD. A study performed by Faulc and co-authors in an animal model with established CKD proved a Klotho-independent causal role for FGF 23 in the pathogenesis of LVH and suggested that chronically elevated FGF 23 levels contributes directly to high rates of

LVH and mortality in individuals with CKD [12]. Leifheit-Nestler performed a recently published study on myocardial autopsy samples of the left ventricle of HD and transplanted patients. They revealed that enhanced myocardial expression of FGF 23 together with Klotho deficiency strongly correlates with the presence of LVH. Cardiac FGF 23 levels associate with time-averaged serum phosphate levels, up-regulation of FGF 23 receptor 4 (FGFR4) and activation of the calcineurin-NFAT signalling pathway, an established mediator of cardiac remodelling and LVH. These alterations are specific for HD patients and not observed in kidney transplant patients [23]. In a study of chronic HD patients performed by Hsu and co-authors, FGF 23 was independently associated with LVH but was not a predictor for a short-term prognosis on 2-year follow-up for mortality [24]. In contrast, our study found that MMI was an important predicting factor for new cardiovascular events in patients without pre-existing CVD, but was not associated with higher FGF 23 levels.

Increasing evidence suggests that VC is one of the most important factors for outcomes of HD patients. VC is

actively regulated process. One of the pivotal calcification inhibitors is now considered MGP which is produced by vascular smooth muscle cells and acts locally on the vascular wall [25]. MGP is a powerful vascular wall-based inhibitor of VC that requires vitamin K-dependent post-translational modification to be fully active. HD patients exhibit reduced vitamin K intake [26] and uraemia interferes with vitamin K recycling [27]. Levels of dephosphorylated uncarboxylated MGP (dp-ucMGP), the inactive form of MGP, increase with chronic kidney disease stage [28].

Studies demonstrated an inverse association between total uncarboxylated MGP and VC in HD patients [8, 9]. Lower levels of circulating dp-cMGP may serve as a predictor of mortality in dialysis patients [29].

As most of the studies primarily focused on the impact of MGP on VC in haemodialysis patients, there is no data about possible direct associations with CVD as it is described in study of Dalmeijer et al. This extended epidemiological study showed that higher circulating desphospho-uncarboxylated MGP (dp-ucMGP) levels were significantly associated with a higher risk of CVD and heart failure. But cir-

culating desphospho-carboxylated MGP and circulating total-uncarboxylated MGP levels were not associated with the risk in type 2 diabetic patients suggesting that poor vitamin K status, that is important in activation of MGP, is associated with increased cardiovascular risk [30]. It is known that compared to healthy individuals, haemodialysis patients have a poor overall vitamin K status [26], but vitamin K deficiency is thought to be a modifiable cardiovascular risk factor [31]. We had no possibility to evaluate vitamin K status and different forms of MGP more precisely, which, in our opinion, is the most significant limitation of the study. Relatively small number of patients, as well as observational and cross-sectional nature of the study, limited the analysis as well.

CONCLUSION

In conclusion, we report a novel association of low FGF 23 and MGP levels and incident cardiovascular events in haemodialysis patients without pre-existing cardiovascular diseases. ♦

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Santrauka

FIBROBLASTŲ AUGIMO FAKTORIAUS 23, MATRIKSO GLA BALTOMO IR NAUJAI ATSIDRUSIŲ ŠIRDIES IR KRAUJAGYSLIŲ LIGŲ SĄSAJOS DIALIZUOJAMIEMS LIGONIAMS, ANKSČIAU NESIRGUSIEMS ŠIOMIS LIGOMIS: VIENO CENTRO KOHORTINIS TYRIMAS

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Įvadas. Galutinės stadijos inkstų nepakankamumu sergantiems ligoniams, gydomiems pakaitine inkstų terapija, širdies ir kraujagyslių ligų bei bendro mirtingumo rizika yra daug didesnė, palyginti su bendra populiacija. Ankstesniuose darbuose nustatėme, kad kraujagyslių kalkėjimas yra vienas svarbiausių rizikos veiksnių, lemiančių didesnę širdies ir kraujagyslių ligų riziką dializuojamiems ligoniams. Literatūros duomenimis, fibroblastų augimo faktorius 23 (FGF 23) ir matrikso Gla baltymas (MGP) gali būti svarbūs tiek kraujagyslių kalkėjimui, tiek ilgalaikėms dializuojamų ligonių

baigtims. Galimos sąsajos tarp minėtų biožymenų bei širdies ir kraujagyslių ligų jau buvo nagrinėtos, tačiau dializėmis gydomi ligoniai į tyrimus nebuvo įtraukti.

Darbo tikslas. Įvertinti galimas sąsajas tarp biožymenų (FGF 23, MGP) ir naujai atsiradusių širdies ir kraujagyslių ligų dializuojamiems pacientams.

Tiriamieji ir metodai. Programinėmis dializėmis gydomi 75 pacientai buvo stebėti atliekant perspektyvų vieno centro tyrimą. Įvertinus kraujagyslių kalkėjimą rentgeno metodu, surinkus klininius ir laboratorinius duomenis, ištyrus FGF 23 ir MGP kiekius kraujyje, ligoniai buvo ste-

bėti ir jiems registruotos širdies ir kraujagyslių ligos. Detaliai analizei visi ligoniai buvo suskirstyti į dvi grupes: ligonių, kuriems širdies ir kraujagyslių ligos jau buvo nustatytos anksčiau, ir ligonių, kuriems šios ligos iki tyrimo nebuvo diagnozuotos.

Rezultatai. Nei biožymenų kiekių kraujyje, nei širdies echoskopijos rezultatams nesiskyrė lyginant ligonius, kuriems iš anamnezės buvo žinomos širdies ir kraujagyslių ligos, su ligoniais, kuriems tokių ligų nebuvo. Atskirai analizuodami grupę ligonių, kurie, anamnezės duomenimis, nesirgo širdies ir kraujagyslių ligomis, nustatėme, kad santykinė šių ligų atsiradimo rizika buvo 5,5 karto didesnė, jei FGF 23 lygis buvo žemesnis nei 41 ng/l ($p=0,008$; 95 % PI 1,6–19,7), ir 5,9 karto didesnė, jei MGP buvo žemesnis nei 1,64 ng/ml ($p=0,006$; 95 % PI 1,7–21).

Išvados. Mažas FGF 23 ir MGP kiekis dializuojamų ligonių kraujyje buvo susijęs su didesne rizika susirgti širdies ir kraujagyslių ligomis.

Reikšminiai žodžiai: dializė, biožymenys, širdies ir kraujagyslių ligos.

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