Srp Arh Celok Lek. 2016 Nov-Dec;144(11-12):608-614
ОРИГИНАЛНИ РАД / ORIGINAL ARTICLE
DOI: 10.2298/SARH1612608P
UDC: 616.61-06; 616.12-084

Prevalence and risk factors of vascular calcification in pre-dialysis patients with Balkan endemic nephropathy

Nenad Petković[1], Radmil Marić[2], Radoslav Gajanin[3], Danijela Batinić[2], Mirjana Ćuk[2], Siniša Ristić[2], Ljubica Djukanović[4]

[1]Fresenius Medical Care Dialysis Center, Šamac, Republic of Srpska, Bosnia and Herzegovina;
[2]University of East Sarajevo, Faculty of Medicine, Foča, Republic of Srpska, Bosnia and Herzegovina;
[3]University of Banja Luka, Faculty of Medicine, Department of Pathology, Banja Luka, Republic of Srpska, Bosnia and Herzegovina;
[4]University of Belgrade, School of Medicine, Belgrade, Serbia

SUMMARY

Introduction Vascular calcifications (VC) are common in patients with chronic kidney disease and present one of manifestations of mineral and bone disorders in these patients.

Objective The aim of this pilot study was to examine the prevalence and risk factors of VC in pre-dialysis patients with Balkan endemic nephropathy (BEN) and other kidney diseases.

Methods The study involved 32 pre-dialysis patients, 15 with BEN and 17 with other kidney diseases. All the patients underwent an interview, objective examination, routine laboratory analyses and measurement of serum concentration of intact parathyroid hormone (iPTH), 25-hydroxyvitamin D3 [25(OH)D3] and osteopontin. VCs in iliac, femoral, radial, and digital arteries were evaluated and Adragao VC score was calculated. The samples of radial artery were collected during the first creation of an arteriovenous fistula, and expression of osteocalcin, bone morphogenic protein-2 osteopontin, and matrix Gla-protein in arterial wall were examined.

Results Patients with BEN were significantly older (71.1 ± 6.1 vs. 54.7 ± 11.1 years), but they had significantly lower systolic and mean blood pressure (95.7 ± 13.2 mmHg vs. 104.3 ± 7.4 mmHg) and lower serum concentration of phosphorus (1.32 ± 0.36 mmol/l vs. 1.65 ± 0.35 mmol/l) and cholesterol (4.3 ± 1.1 mmol/l vs. 5.2 ± 0.8 mmol/l) than patients with other kidney diseases. Mean VC score was significantly lower in patients with BEN than in those with other kidney diseases (2.8 ± 1.7 vs. 4.6 ± 1.8; p = 0.009), but expression of four examined proteins in arterial wall differed insignificantly between the two groups. VC score correlated significantly with serum concentrations of cholesterol, triglycerides (positively), and iPTH (negatively).

Conclusion Pre-dialysis BEN patients had a significantly lower mean score of VC than patients with other kidney diseases.

Keywords: vascular calcification; pre-dialysis patients; Balkan endemic nephropathy

INTRODUCTION

Balkan endemic nephropathy (BEN) is a familial, chronic, tubule-interstitial disease that occurs in limited areas of the Balkan Peninsula. The disease is usually without symptoms, progresses slowly and today is mainly revealed in the sixth decade of life [1, 2]. Therefore, the diagnosis is usually reached in the advanced stage of the disease when tubular disorders, metabolic disorders characteristic for chronic renal failure including mineral and bone disorders as well as moderate hypertension and anemia are also present.

Vascular calcifications (VC) are common in patients with chronic kidney disease and represent a significant predictor of both general and cardiovascular mortality [3, 4, 5]. Pathogenesis of VC has been the subject of many studies that have found its association with mineral metabolism disorders and renal bone disease [5, 6]. Today, it is considered that VC is not the result of passive deposition of calcium phosphate, but the result of an active process similar to the process of bone calcification. This process presents a series of complex biochemical and cellular events in which a number of regulatory proteins that induce or inhibit the deposition of minerals in the blood vessels actively participate [4–7].

In BEN as chronic tubule-interstitial disease hyperphosphatemia, one of the main pathogenic factors of VC is uncommon even in patients on regular hemodialysis [8]. It could be proposed that the prevalence of VC in BEN is lower than in other diseases, although there is no published information on this topic.

OBJECTIVE

The aim of this pilot study was to compare prevalence of VC and factors that may contribute to their development in pre-dialysis patients with BEN and other kidney disease.

Correspondence to:
Nenad PETKOVIĆ
Fresenius Medical Care Dialysis Center
Cara Dušana 70
76230 Šamac
Republic of Srpska
Bosnia and Herzegovina
nenad.petkovic@fmc-ag.com

METHODS

Patients

The study involved 32 patients in the fifth stage of chronic kidney disease before starting dialysis treatment, examined at the moment of creation of arteriovenous fistula. The patients were divided into two groups; the first consisted of 15 patients with BEN as primary kidney disease, and the second of 17 patients with other kidney diseases (glomerulonephritis in six, diabetic nephropathy in four, pyelonephritis in four, and three patients with other kidney diseases). During the interview and objective examination, the following data of all the patients were registered: age, sex, duration of chronic kidney disease, and a risk factor of cardiovascular diseases (hypertension, diabetes mellitus, dyslipidemia, and smoking), body mass index, blood pressure.

Laboratory analyses

Hemoglobin level as well as serum concentrations of calcium, phosphorus, alkaline phosphatase, iron, total cholesterol, LDL cholesterol, and triglycerides were measured by standard laboratory methods. Serum concentration of intact parathyroid hormone (iPTH) was measured by immunochemiluminescent method on Cobas 6000 modular (Roche Diagnostics International Ltd., Rotkreuz, Switzerland) (normal value: 15–65 pg/ml), 25-hydroxyvitamin D3 [25(OH) D3] by electrochemiluminescence immunoassay – ECLIA on Cobas 6000 modular (normal value: 75–250 ng/ml) and osteopontin by ELISA technique using sandwich human osteopontin ELISA kit (Abcam plc., Cambridge, United Kingdom) and Microplate Reader RT-2100 C (Rayto Life and Analytical Sciences CO. Ltd., Shenzhen, China) (normal value ≥5 ng/ml). Glomerular filtration rate (eGFR) was estimated by the MDRD equation: eGFR = 175 × serum-creatinine$^{-1.154}$ × age$^{-0.203}$ × 0.742 (women) [9].

Radiological examinations

VCs in the iliac, femoral, radial, and digital arteries were evaluated by one radiologist (S.R.) in plain radiographic films of pelvis and hands in all examined patients. A simple VC score was calculated as described by Adragao et al. [10]. In brief, radiographic films of the pelvis were divided into four sections by two lines – a horizontal line over the upper limit of both femoral heads, and a vertical line along the middle of the vertebral column. The films of each hand were divided by a horizontal line over the upper limit of the metacarpal bones. The presence of linear calcifications in each part of the film was counted as 1, and its absence as 0. The VC score was the sum of scores in all parts of films ranging from 0 to 8. VC were quantified as 0 = no calcification, 1–3 = mild calcification, and ≥4 severe calcification.

Histopathological analysis

A sample of radial artery, 3–5 mm in length, was collected during the first creation of arteriovenous fistula for hemodialysis access. The sample was fixed in buffered formalin for 12–24 hours, then processed and embedded in paraffin. The sections of 5 μm were used for immunohistochemical analysis done by an experienced pathologist blinded to the clinical data. To demonstrate the expression of osteocalcin, bone morphogenic protein-2 (BMP-2), osteopontin and matrix Gla-protein (MGP) in the wall of the artery, immunohistochemical staining with the following human-specific antibodies was used: rabbit polyclonal antibodies to osteocalcin, BMP-2, and MGP, and rabbit monoclonal antibody to osteopontin, all produced by Abcam, Cambridge, United Kingdom. Protein expression was quantified using a semi-quantitative scoring system (0 = no expression; 1 = weak or moderate focal or diffuse weak expression; 2 = high focal or moderate diffuse expression; 3 = high diffuse expression) in high-power fields (×200 magnification). Scores of 0 and 1 were considered to be a negative finding, and scores 2 and 3 positive.

Statistical analysis

Continuous variables are presented as mean and standard deviation, and categorical as frequencies. The Student's t-test was used to assess differences between continuous variables and the χ^2 and Fisher's exact test between categorical variables, as appropriate. Correlation between continuous variables was calculated using Pearson's linear correlation coefficient, and correlation between categorical variables by Cramer's phi coefficient. All analyses were performed using the SPSS Statistics for Windows, Version 21.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Table 1 presents the main characteristics of the patients studied. Patients with BEN were significantly older than patients with other kidney diseases and had significantly lower systolic blood pressure as well as mean blood pressure (95.7 ± 13.2 mmHg vs. 104.3 ± 7.4 mmHg; p = 0.037). All but two patients in each group had hypertension and were treated with antihypertensive drugs, more frequently

Table 1. The basic characteristics of the studied patients

Characteristics	Patients		p
	With BEN	With other kidney diseases	
Number of patients	15	17	
Sex – male	11	9	0.234
Age (years)	71.7 ± 6.1	54.7 ± 11.1	<0.0001
BMI (kg/m^2)	25.0 ± 3.2	24.8 ± 5.1	0.903
Systolic BP (mmHg)	133.5 ± 22.3	151.5 ± 14.5	0.014
Diastolic BP (mmHg)	76.9 ± 8.9	79.2 ± 8.1	0.439
Duration of CKD (years)	3.5 ± 2.5	4.1 ± 2.8	0.543
$CaCO_3$ (No. of patients treated)	11	13	0.838
Calcitriol (No. of patients treated)	15	17	1.0

BEN – Balkan endemic nephropathy; BMI – body mass index; BP – blood pressure; CKD – chronic kidney disease

Table 2. Comparison of laboratory findings between patients with Balkan endemic nephropathy (BEN) and other kidney diseases

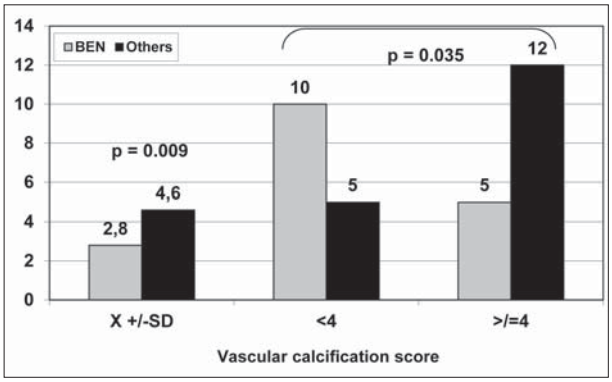
Laboratory findings	Patients		p
	with BEN	with other kidney diseases	
eGFR-MDRD (ml/min/1.73 m^2)	14.5 ± 2.8	7.5 ± 1.5	0.759
Calcium (mmol/l)	2.40 ± 0.24	2.33 ± 0.15	0.298
Phosphorus (mmol/l)	1.32 ± 0.36	1.65 ± 0.35	0.015
Ca × P (mmol^2/l^2)	3.19 ± 0.98	3.81 ± 0.78	0.059
Alkaline phosphatase (U/l)	74.5 ± 25.4	86.2 ± 22.7	0.179
PTH (pg/ml)	188.6 ± 105.4	150.0 ± 115.6	0.331
Vitamin D (ng/ml)	51.1 ± 22.1	53.0 ± 23.1	0.809
Osteopontin (ng/ml)	59.2 ± 32.5	87.3 ± 82.9	0.211
Cholesterol (mmol/l)	4.3 ± 1.1	5.2 ± 0.8	0.049
LDL cholesterol (mmol/l)	2.4 ± 0.8	2.6 ± 0.6	0.457
Triglycerides (mmol/l)	1.9 ± 0.9	2.3 ± 0.8	0.134
Hemoglobin (g/l)	114.3 ± 10.5	116.1 ± 8.1	0.575
Iron (μmol/l)	13.6 ± 5.3	15.3 ± 6.6	0.446

eGFR – estimated glomerular filtration rate; MDRD – Modification of Diet in Renal Disease; PTH – parathormone; LDL – low-density lipoprotein

with angiotensin-converting enzyme inhibitors. The differences between the two groups in body mass index, diastolic blood pressure, and duration of chronic kidney disease were not significant.

The results of laboratory analyses are presented in Table 2. There was no significant difference in eGFR between the two groups. Patients with BEN had a significantly lower serum concentration of phosphorus and cholesterol than patients with other kidney diseases, while the difference in the product of phosphorus and calcium was on the border of statistical significance. No statistical significance was found between the two groups in other laboratory findings. It should be noted that although mean serum calcium concentration was near the upper limit of target range proposed by The National Kidney Foundation's Kidney Disease Outcomes Quality Initiative (KDOQI) guidelines [11], analysis of individual values showed that about one third of patients had mean serum calcium concentration above the target range (5/15 of BEN patients, and 6/17 of others). Serum iPTH concentration was similar in both groups and about one half of patients of both groups had iPTH concentration below 150 pg/ml, the lower limit of KDOQI target range (seven BEN patients and nine others).

Graph 1 shows the score of VC estimated in X-ray films of hands and pelvis. When compared the number of patients with a score of VC less than or equal to 4 and greater than 4, it appeared that 10/15 of patients with BEN had score below 4, while this was the case with only 5/17 of patients with other kidney diseases. Pearson's χ^2 test showed that this difference was statistically significant. In addition, patients with BEN had a significantly lower mean score of VC (2.8 ± 1.7) than patients with other kidney disease (4.6 ± 1–8; p = 0.009). Expression of four examined proteins in arterial wall differed insignificantly between the two groups of patients: 3/13 of BEN patients and 1/14 of patients with other kidney disease had an expression score equal to or greater than 2 for osteocalcin; 1/13 of BEN and 1/14 of others for BMP; 6/13 of BEN and 5/14 of others for osteopontin; and 3/13 of BEN and 5/14 of other patients for MGP.



Graph 1. Vascular calcification score in patients with Balkan endemic nephropathy (BEN) and other kidney diseases

Patients of both groups were divided into two subgroups – one with a VC score equal or less than 4, and the other with the score greater than 4. Characteristics of these subgroups were compared and are presented in Table 3. The number of BEN patients with VC score below 4 was significantly higher compared to patients with other kidney disease. Patients who had VC score below 4 had statistically significant lower concentrations of total cholesterol and triglyceride but higher concentration of iPTH, although the results bordered statistical significance. No significant difference was found between the patients with VC score less than 4 and equal to or greater than 4 in the expression of osteocalcin, BMP-2, osteopontin, and MGP in the wall of the radial artery.

Six patients from each group had a positive history of angina pectoris, signs of ischemia on ECG were recorded in seven patients with BEN and in nine patients with other kidney diseases, but only among patients with other kidney diseases three patients had a history of myocardial infarctions. Also, the number of smokers was higher in the group with other kidney diseases than in the BEN group, i.e. five vs. two patients. However, all these differences did not reach statistical significance.

Table 4 presents the results of correlation between VC score and all demographic, clinical, and laboratory variables determined in both groups. Only variables that correlated significantly with VC score are presented. Pearson's linear correlation coefficient showed a significant positive correlation between VC score and serum concentration of cholesterol and triglycerides and significant negative correlation with serum iPTH concentration. It is interesting that between body mass index and VC score a negative, but insignificant, correlation was found (r = -0.326; p = 0.069). Cramer's phi coefficient used to investigate the correlation between categorical variables showed significant correlation between VC score and presence of peripheral vascular disease.

DISCUSSION

This study showed that patients with BEN had a significantly lower mean score of VC than patients with other kidney diseases. The number of patients with BEN who

doi: 10.2298/SARH1612608P

Table 3. Comparison of patients with vascular calcification score less than 4 and equal to or greater than 4

Parameter		Vascular calcification score		p
		<4	≥4	
Sex	Male	10	10	0.647
	Female	5	7	
Age (years)		65.3 ± 9.0	60.4 ± 14.6	0.263
Diagnosis	BEN	10/15	5/15	0.035
	Other	5/17	12/17	
eGFR (ml/min/1.73 m^2)		8.3 ± 2.3	6.9 ± 1.9	0.075
Cholesterol (mmol/l)		4.3 ± 1.0	5.3 ± 1.0	0.01
Triglycerides, mmol/l		1.6 ± 0.5	2.6 ± 0.9	0.001
S-iron		12.6 ± 5.5	16.2 ± 6.1	0.093
PTH (pg/ml)		208.0 ± 135.6	132.9 ± 70.2	0.051
S-calcium >2.4 (mmol/l)		6/15	5/17	0.720
S-phosphorus >1.8 (mmol/l)		2/15	3/15	0.714
Expression score	Osteocalcin ≥2	1/12	3/15	0.762
	BMP-2 ≥2	0/12	2/15	0.565
	Osteopontin ≥2	3/12	8/15	0.274
	MGP ≥2	3/12	5/15	0.962

Patients of both groups are included in this analysis.
eGFR – estimated glomerular filtration rate; BMP – bone morphogenic protein, MGP – matrix Gla-protein
Expression score – expression of listed proteins in the radial artery wall quantified semi-quantitatively as described in Methods.

Table 4. Variables that significantly correlate with vascular calcification score

Parameter	Pearson's coefficient of linear correlation	
	r	p
Cholesterol (mmol/l)	0.444	0.011
Triglycerides (mmol/l)	0.520	0.002
PTH (pg/ml)	-0.394	0.026
Parameter	Cramer's phi coefficient	
	Value	p
Peripheral VD	0.373	0.038

had a VC score less than 4 was significantly higher than that in patients with other kidney diseases. Analysis of factors that could be associated with the development of VC showed that patients with BEN had a significantly lower systolic and mean blood pressure, as well as serum concentration of cholesterol and phosphorus, lower product of phosphorus and calcium (on the borderline of statistical significance), but were significantly older than patients with other diseases. Significant positive correlation was found between VC score and serum concentration of cholesterol and triglycerides, but negative with serum iPTH concentration.

Numerous risk factors are associated with VC in chronic kidney disease. In addition to "traditional" risk factors, such as age, male sex, hypertension, diabetes, and dyslipidemia, "non-traditional" factors, such as uremic toxins, disorders of mineral metabolism and their regulatory hormones (PTH, vitamin D), excessive use of calcium salts as phosphate binders, inflammation, malnutrition, and oxidative stress substantively participate in the VC development [12]. BEN patients included in the present study were significantly older than patients with other kidney diseases and it was the only risk factor for VC that was more pronounced in patients with BEN than in others. Many authors reported that the age of patients with manifested BEN has shifted to the older ages [13, 14]. While most of the patients in the first descriptions of the disease were in the fourth decade of life [13, 15], in 1980s, most patients with manifested disease were in their sixth decade [1, 2]. Although age is a well known risk factor for VC, our BEN patients, in spite of older age than those with other kidney diseases, had less VC. However, several other risk factors for VC were less pronounced in patients with BEN than in those with other kidney diseases: BEN patients had significantly lower systolic and mean blood pressure, lower serum concentration of cholesterol and phosphorus than patients with other kidney diseases, and the difference in product of phosphorus and calcium between the groups was on the borderline of statistical significance. Some of these characteristics of BEN have already been described and our results confirmed these findings. Thus, in contrast to the earlier studies that reported usually normal blood pressure in patients with BEN [15], recent studies have shown that the prevalence of hypertension in BEN patients is similar to those with other kidney diseases but it is easier to regulate [16, 17, 18]. In our studies, all but two patients in each group had hypertension, which was, however, better regulated by antihypertensive drugs in BEN patients.

The present study also showed significantly lower serum concentration of cholesterol in BEN patients compared to patients with other kidney diseases. Pavlović et al. [19] found significantly lower total cholesterol and free cholesterol serum concentration in BEN family members than in members of non-BEN families living in the same location and healthy controls living outside the BEN region. The authors explained this lower concentration of cholesterol by lecithin-cholesterol acyltransferase deficiency found in BEN family members.

In addition to lower values of the two abovementioned risk factors for VC (blood pressure and serum cholesterol concentration), BEN patients also had a significantly lower serum phosphorus concentration in comparison to patients with other kidney diseases. The significantly lower serum phosphorus concentration in BEN patients led to lower production of calcium and phosphorus in BEN patients. Disorders of mineral metabolism present the most important pathogenic factors for VC in chronic kidney disease [20]. Experimental studies have shown that increasing phosphorus concentrations can induce human arterial vascular smooth muscle cells to transdifferentiate towards an osteoblastic phenotype [5, 21]. There is little data on mineral disorders in BEN. Bukvić et al. [8] were the only ones who described that hyperphosphatemia appeared very rarely in BEN patients on hemodialysis and that normal serum phosphorus concentrations in BEN patients were maintained even without the use of phosphate binders. Normal and low phosphorus level is uncommon in patients in advanced stages of chronic kidney disease. It can be caused by chronic tubular disorders and increased phosphate excretion, but also by malabsorption of phosphorus or phosphate binders overdosage [22, 23, 24]. Although we have no evidence, we can assume the increased phosphate excretion in our BEN patients as the cause of

lower phosphorus concentration. The majority of patients of both groups used calcium carbonate as phosphate binders but in similar moderate doses (BEN: 2.8 ± 0.51 g/day; others: 3.1 ± 0.81 g/day; p = 0.227). The use of calcium carbonate, the only available phosphate binder in our country, was one of the factors that caused serum calcium concentration above target range proposed by KDOQI guidelines in one third of the examined patients. This was undoubtedly one of the factors that contributed to the fact that the mean iPTH level in both groups was near the lower limit of KDOQI target guideline range for iPTH, and that about one half of patients of both groups had iPTH below the target range. Proportion of patients with iPTH below the target range is higher in our patients than in patients involved in several European studies, but significantly lower percentage of their patients used calcium-based phosphate binders [25, 26]. Comparative studies showed that the use of calcium-based phosphate binders is more frequently associated with episodes of hypercalcemia and low iPTH level than the use of calcium-free phosphate binders [27]. Low iPTH is one of risk factors for the development of VC and low bone turnover [28, 29, 30]. KDOQI guidelines suggest that, if the intact PTH levels fall below the lower target limit, calcitriol, vitamin D analogs, and/or calcimimetics should be reduced or stopped. All this suggests that in our limited conditions a careful follow-up of serum calcium and phosphorus concentration is required in order to maintain iPTH in the target range.

Our investigations of risk factors for VC were complemented with measuring serum osteopontin concentration, one of the inhibitors of extraskeletal calcification whose effect is independent of serum phosphorus and alkaline phosphatase concentration [30]. Also, the expression of osteocalcin, BMP-2, osteopontin, and MGP in the wall of the radial artery was examined. The results presented showed that there was no statistically significant difference in the serum concentration of osteopontin in patients with BEN and those with other kidney diseases. However, patients with BEN had a lower serum concentration of osteopontin than others, and in the radial artery of BEN patients osteopontin was expressed in six samples, while the expression of other examined proteins was found in less than three samples (data not presented). Nevertheless, no significant difference was found between the patients with VC score <4 and ≥4 in the expression of all four proteins in the wall of the radial artery. The recent analysis performed on the Amiens CKD database on VC biomarkers found traditional cardiovascular risk factors as a reliable predictor of VC but not new stimulators and inhibitors of VC previously suggested as major participants in VC in experimental studies [31]. In addition, several studies found traditional risk factors and serum concentration of calcium and phosphorus as the main risk factors for VC in patients with diabetes [32, 33]. Our results are in accordance with these data showing significant positive correlation between VC score and serum concentration of cholesterol and triglycerides and significant negative correlation with serum iPTH, regulatory hormone of mineral metabolism.

CONCLUSION

Patients with BEN had a significantly lower mean score of VC than patients with other kidney diseases. Traditional risk factors and mineral disorders were found as the main risk factors for VC and patients with BEN had significantly less pronounced three of these factors – lower systolic blood pressure, lower serum cholesterol, and phosphorus concentration, but insignificantly lower product of calcium and phosphorus than patients with other kidney diseases. A significant difference in VC score between BEN patients and those with other kidney diseases obtained in this pilot study requires to be confirmed in a larger, multicenter study.

ACKNOWLEDGMENT

This work was supported by the Ministry of Science and Ecology of the Republic of Serbia, contract No. 175025.

REFERENCES

1. Radovanović Z, Sindjić M, Polenaković M, Djukanović Lj, Petronić V, editors. Endemic Nephropathy. Belgrade: Institute for Textbooks and Teaching Aids; 2000.
2. Bukvic D, Jankovic S, Maric I, Stosovic M, Arsenovic A, Djukanovic Lj. Today endemic nephropathy is disease of the elderly with a good prognosis. Clin Nephrol. 2009; 72(2):105–13. [PMID: 19640367]
3. Goodman W, Goldin J, Kuizon B, Yoon C, Gales B, Sider D, et al. Coronary-artery calcification in young adults with end-stage renal disease who are undergoing dialysis. N Engl J Med. 2000; 342(20):1478–83. [DOI: 10.1056/NEJM200005183422003] [PMID: 10816185]
4. Schlieper G, Schurgers L, Brandenburg V, Reutlingsperger C, Floege J. Vascular calcification in chronic kidney disease: an update. Nephrol Dial Transplant. 2016; 31(1):31–9. [DOI: 10.1093/ndt/gfv111] [PMID: 25916871]
5. Jono S, McKee MD, Murry CE, Shioi A, Nishizawa Y, Mori K, et al. Phosphate regulation of vascular smooth muscle cell calcification. Circ Res. 2000; 87:E10–7. [DOI: 10.1161/01.RES.87.7.e10] [PMID: 11009570]
6. London GM, Marty C, Marchais SJ, Guerin AP, Metivier F, de Vernejoul MC. Arterial calcifications and bone histomorphometry in end-stage renal disease. J Am Soc Nephrol. 2004; 15(7):1943–51. [DOI: 10.1097/01.ASN.0000129337.50739.48] [PMID: 15213285]
7. Ketteler M, Rothe H, Krüger T, Biggar PH, Schlieper G. Mechanisms and treatment of extraosseous calcification in chronic kidney disease. Nat Rev Nephrol. 2011; 7(9):509–16. [DOI: 10.1038/nrneph.2011.91] [PMID: 21769106]
8. Bukvić D, Stefanović D, Milić M, Marić I, Djukanović Lj. Hyperphosphatemia Appears Infrequently in Balkan Endemic Nephropathy Patients on Maintenance Hemodialysis. BANTAO J. 2003; 1:108–10.
9. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: A new prediction equation. Modification of diet in renal disease study group. Ann Intern Med. 1999; 130(6):461–70. [DOI: 10.7326/0003-4819-130-6-199903160-00002] [PMID: 10075613]
10. Adragao T, Pires A, Lucas C, Birne R, Magalhaes L, Gonçalves M, et al. A simple vascular calcification score predicts cardiovascular risk in haemodialysis patients. Nephrol Dial Transplant. 2004; 19(6):1480–8. [DOI: 10.1093/ndt/gfh217] [PMID: 15034154]

11. National Kidney Foundation. K/DOQI Clinical Practice Guidelines for Bone Metabolism and Disease in Chronic Kidney Disease. Am J Kidney Dis. 2003; 42(suppl 3):S1–S202. [DOI: 10.1016/S0272-6386(03)00905-3] [PMID: 14520607]
12. Moe S, Chen N. Mechanisms of vascular calcification in chronic kidney disease. Am Soc Nephrol. 2008; 19:213–6. [DOI: 10.1681/ASN.2007080854] [PMID: 18094365]
13. Janković S, Bukvić D, Marinković J, Janković J, Marić I, Djukanović L. Time trends in Balkan endemic nephropathy incidence in the most affected region in Serbia, 1977–2009: the disease has not yet disappeared. Nephron Dial Transplant. 2011; 26:3171–6. [DOI: 10.1093/ndt/gfr059] [PMID: 21355065]
14. Cvitković A, Vuković-Lela I, Edwards KL, Karanović S, Jurić D, Cvorišćec D, et al. Could disappearance of endemic (Balkan) nephropathy be expected in forthcoming decades? Kidney Blood Press Res. 2012; 35(3):147–52. [DOI: 10.1159/000333836] [PMID: 22116163]
15. Danilović V, Djurišić M, Mokranjac M, Stojimirović B, Živojinović J, Stojaković P. Néphrites chroniques provoquées par l'intoxication au plomb par voie digestive (farine). Presse méd. 1957; 65(90):2039–40. [PMID: 13505667]
16. Arsenovic A, Bukvic D, Trbojevic S, Maric I, Djukanovic L. Detection of renal dysfunctions in family members of patients with Balkan Endemic Nephropathy. Am J Nephrol. 2005; 25(1):50–4. [DOI: 10.1159/000084105] [PMID: 15731549]
17. Dimitrov P, Tsolova S, Georgieva R, Bozhilova D, Simeonov V, Bonev A, et al. Clinical markers in adult offspring of families with and without Balkan endemic nephropathy. Kidney Int. 2006; 69(4):723–9. [DOI: 10.1038/sj.ki.5000120] [PMID: 16407881]
18. Ristić S, Lukić L, Maksimović Z, Marić S, Marić V, Kovačević M, et al. High prevalence of risk factors for chronic kidney disease in Balkan endemic nephropathy foci. Ren Fail. 2012; 34(4):467–71. [DOI: 10.3109/0886022X.2012.656564 [PMID: 22364394
19. Pavlović NM, Orem WH, Tatu CA, Lerch HE, Bunnell JE, Feder GL, et al. The role of lecithin cholesterol acyltransferase and organic substances from coal in the etiology of Balkan endemic nephropathy: a new hypothesis. Food Chem Toxicol. 2008; 46(3):949–54. [DOI:10.1016/j.fct.2007.10.033] [PMID: 18063285]
20. Shroff RC, McNair R, Skepper JN, Figg N, Schurgers LJ, Deanfield J, et al. Chronic mineral dysregulation promotes vascular smooth muscle cell adaptation and extracellular matrix calcification. J Am Soc Nephrol. 2010; 21(1):103–12. [DOI: 10.1681/ASN.2009060640] [PMID: 19959717]
21. Crouthamel MH, Lau WL, Leaf EM, Chavkin NW, Wallingford MC, Peterson DF, et al. Sodium-dependent phosphate cotransporters and phosphate-induced calcification of vascular smooth muscle cells: redundant roles for PiT-1 and PiT-2. Arterioscler Thromb Vasc Biol. 2013; 33(11):2625–32. [DOI: 10.1161/ATVBAHA.113.302249] [PMID: 23968976]
22. Laroche M. Phosphate, the renal tubule, and the musculoskeletal system. Joint Bone Spine. 2001; 68(3):211–5. [DOI: 10.1016/S1297-319X(01)00274-3] [PMID: 11394620]
23. Wiegmann TB, Kaye M. Malabsorption of calcium and phosphate in chronic renal failure: ^{32}P and ^{45}Ca studies in dialysis patients. Clin Nephrol. 1990; 34(1):35–41. [PMID: 2387101]
24. Delmez JA, Fallon MD, Harter HR, Hruska KA, Slatopolsky E, Teitelbaum SL. Does strict phosphorus control precipitate renal osteomalacia? J Clin Endocrinol Metab. 1986; 62(4):747–52. [DOI: 10.1210/jcem-62-4-747] [PMID: 3949954]
25. Fernández-Martín JL, Carrero JJ, Benedik M, Bos WJ, Covic A, Ferreira A, et al. COSMOS: the dialysis scenario of CKD-MBD in Europe. Nephrol Dial Transplant. 2013; 28(7):1922–35. [DOI: 10.1093/ndt/gfs418] [PMID: 23166310]
26. Górriz JL, Molina P, Bover J, Barril G, Martín-de Francisco AL, Caravaca F, et al. En nombre de los investigadores del estudio OSERCE. Characteristics of bone mineral metabolism in patients with stage 3-5 chronic kidney disease not on dialysis: results of the OSERCE study. Nefrologia. 2013; 33(1):46–60. [DOI: 10.3265/Nefrologia.pre2012.Nov.11703] [PMID: 23364626]
27. Spaia S. Phosphate binders: Sevelamer in the prevention and treatment of hyperphosphataemia in chronic renal failure. Hippokratia. 2011; 15(Suppl1):22–6. [PMID: 21897754]
28. Moe SM. Vascular calcification and renal osteodystrophy relationship in chronic kidney disease. Eur J Clin Invest. 2006; 36(Suppl.2):51–62. [DOI: 10.1111/j.1365-2362.2006.01665.x] [PMID: 16884398]
29. Kakuta T, Tanaka R, Hyodo T, Suzuki H, Kanai G, Nagaoka M, et al. Effect of sevelamer and calcium-based phosphate binders on coronary artery calcification and accumulation of circulating advanced glycation end products in hemodialysis patients. Am J Kidney Dis. 2011; 57(3):422–31. [DOI: 10.1053/j.ajkd.2010.10.055] [PMID: 21239096]
30. Checheriță IA, David C, Stoica L, Popescu P, Ciocalteu A, Lascar I. New mediators of vascular damage in dialysed patients. Rom J Morphol Embryol. 2011; 52(2):533–6. [PMID: 21655639]
31. Liabeuf S, Okazaki H, Desjardins L, Fliser D, Goldsmith D, Covic A, et al. Vascular calcification in chronic kidney disease: are biomarkers useful for probing the pathobiology and the health risks of this process in the clinical scenario? Nephrol Dial Transplant. 2014; 29(7):1275–84. [DOI: 10.1093/ndt/gft368] [PMID: 24009287]
32. Taniwaki H, Ishimura E, Tabata T, Tsujimoto Y, Shioi A, Shoji T, et al. Aortic calcification in hemodialysis patients with diabetes mellitus. Nephrol Dial Transplant. 2005; 20(11):2472–8. [DOI: 10.1093/ndt/gfi039] [PMID: 16077143]
33. Mehrotra R, Budoff M, Christenson P, Ipp E, Takasu J, Gupta A, et al. Determinants of coronary artery calcification in diabetics with and without nephropathy. Kidney Int. 2004; 66(5):2022–31. [DOI: 10.1111/j.1523-1755.2004.00974.x] [PMID: 15496175]

Преваленција и фактори ризика васкуларних калцификација код болесника са балканском ендемском нефропатијом у одмаклој хроничној инсуфицијенцији бубрега

Ненад Петковић[1], Радмил Марић[2], Радослав Гајанин[3], Данијела Батинић[2], Мирјана Ћук[2], Синиша Ристић[2], Љубица Ђукановић[4]

[1]Центар за хемодијализу *Fresenius Medical Care*, Шамац, Република Српска, Босна и Херцеговина;
[2]Универзитет у Источном Сарајеву, Медицински факултет, Фоча, Република Српска, Босна и Херцеговина;
[3]Универзитет у Бањој Луци, Медицински факултет, Одељење патологије, Бања Лука, Република Српска, Босна и Херцеговина;
[4]Универзитет у Београду, Медицински факултет, Београд, Србија

КРАТАК САДРЖАЈ

Увод Васкуларне калцификације (ВК) честе су код болесника са хроничним болестима бубрега и представљају једну од манифестација поремећаја метаболизма минерала ових болесника.

Циљ рада Циљ овог рада је био испитивање преваленције и фактора ризика ВК код болесника са балканском ендемском нефропатијом (БЕН) и другим болестима бубрега у терминалној бубрежној инсуфицијенцији пре започињања лечења дијализама.

Методе рада Испитивање је обухватило 32 болесника, 15 са БЕН и 17 са другим болестима бубрега. Поред анамнезе и објективног прегледа свим болесницима су урађене рутинске лабораторијске анализе, мерење концентрације интактног паратхормона (*iPTH*), 25-хидроксивитамина Д3 [*25(OH)D3*] и остеопонтина. ВК у илијачним, феморалним и дигиталним артеријама су процењене по методи *Adragao*. Узорак радијалне артерије узет је током операције прве артериовенске фистуле и у њему је методама имунохистологије испитана експресија остеокалцина, морфогеног протеина кости-2, остеопонтина и Гла-протеина матрикса.

Резултати Болесници са БЕН су били значајно старији (71,1 ± 6,1 вс. 54,7 ± 11,1 година), имали су значајно нижи систолни и средњи артеријски притисак (95,7 ± 13,2 *mm Hg* вс. 104,3 ± 7,4 *mm Hg*), нижу концентрацију фосфора (1,32 ± 0,36 *mmol/l* вс. 1,65 ± 0,35 *mmol/l*) и холестерола у серуму (4,3 ± 1,1 *mmol/l* вс. 5,2 ± 0,8 *mmol/l*) него болесници са другим болестима бубрега. Просечан скор ВК био је значајно мањи код болесника са БЕН у односу на болеснике са другим болестима бубрега (2,8 ± 1,7 вс. 4,6 ± 1,8; $p = 0,009$), али није било значајне разлике у експресији четири испитана протеина и зиду артерије. Утврђена је значајна корелација између скора ВК и концентрације холестерола, триглицерида (позитивна) и *iPTH* (негативна).

Закључак Болесници са БЕН у одмаклој хроничној инсуфицијенцији бубрега имали су значајно нижи скор ВК од болесника са другим болестима бубрега.

Кључне речи: васкуларне калцификације; хронична инсуфицијенција бубрега; балканска ендемска нефропатија

Примљен • Received: 25/08/2015 **Ревизија • Revision:** 13/10/2015 **Прихваћен • Accepted:** 26/10/2015