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Association between serum fetuin-A concentrations and vascular calcifications among patients on maintenance hemodialysis**

Związek pomiędzy stężeniem fetuiny-A w surowicy i zwapnieniami naczyniowymi u chorych leczonych powtarzanymi hemodializami

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Keywords

fetuin-A, IL-6, hsCRP, calcium scoring, intima-media thickness, bone mineral density

Słowa kluczowe

fetuina-A, IL-6, hsCRP, wskaźnik uwapnienia, grubość błony wewnętrznej i środkowej, gęstość mineralna kości

Summary

Introduction. Vascular calcifications in patients with end stage renal disease on maintenance hemodialysis is the leading cause of cardiovascular events and death. Epidemiological studies have shown that severe vascular calcifications and associated increased risk of morbidity and mortality occur particularly in patients with reduced serum fetuin-A levels.

Aim. The aim of the study was to assess the severity of calcifications in the cardiovascular system of patients treated with repeated hemodialysis depending on the concentrations of serum fetuin-A, bone mineral density (BMD) and markers of inflammation.

Material and methods. The study was performed in 71 patients (31 women, 40 men) aged 60 ± 12 years on chronic dialysis because of end-stage renal failure for a period of approximately 75 ± 57.2 months. The level of vascular calcifications was examined based on coronary artery calcification score (CaSc) and common carotid artery intima-media thickness (CCA-IMT). Bone mineral density was measured using Lunar DPX. The serum concentrations of routine laboratory test were performed based on Modular P analyzer (Roche Diagnostics), iPTH using Nichols method, hsCRP and IL-6 using nephelometric techniques while fetuin A, was measured using commercially available ELISA kits.

Results. Densitometry showed a decrease in bone mineral density both within the ranks of the femur (Tscore = -2.10 ± 1.00) and lumbar spine (Tscore = -1.03 ± 1.51). Calcium scoring performed in patients showed severe calcification; CaSc – 511 (158-2394) Agatston units and the CCA-IMT was 0.90 ± 0.42. Fetuin-A concentrations were significantly negatively associated with patients age ($r = -0.26$, $p = 0.04$), log (iPTH) ($r = -0.31$, $p = 0.02$), log (CRP) ($r = -0.31$, $p = 0.02$), log (IL-6) ($r = -0.41$, $p = 0.001$), log (CaSc) ($r = -0.29$, $p = 0.03$) and log (CCA-IMT) ($r = -0.28$, $p = 0.04$). Concentrations of fetuin-A were positively correlated with albumin ($r = 0.37$, $p = 0.003$) and BMD in the region of femoral neck ($r = 0.26$, $p = 0.04$).

Conclusions. Patients on maintenance hemodialysis suffer from severe calcifications of vascular system. Low serum fetuin-A levels are negatively associated with calcification parameters, inflammation and hyperparathyroidism.

Conflict of interest
Konflikt interesów

None
Brak konfliktu interesów

Streszczenie

Wstęp. Zwapnienia naczyniowe u pacjentów ze schyłkową niewydolnością nerek leczonych powtarzanymi hemodializami są główną przyczyną zdarzeń sercowo-naczyniowych i zgonów. W badaniach epidemiologicznych wykazano, że duże zwapnienia naczyniowe i związane z nimi ryzyko chorobowości i śmiertelności występują szczególnie u chorych z niskimi stężeniami fetuiny-A w surowicy.

Cel pracy. Celem badania była ocena nasilenia zwapnień w układzie sercowo-naczyniowym u chorych leczonych powtarzanymi hemodializami w zależności od stężenia w surowicy fetuiny-A, gęstości mineralnej kości (BMD) i wskaźników zapalenia.

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**This paper was dedicated to Prof. Franciszek Kokot, the creator of Polish nephrology, wonderful man, an educator of many generations of doctors with the best wishes.

Materiał i metody. Badania przeprowadzono w grupie 71 pacjentów (31 kobiet, 40 mężczyzn) w wieku 60 ± 12 lat leczonych powtarzanymi dializami z powodu schyłkowej niewydolności nerek przez okres 75 ± 57,2 miesiąca. Nasilenie zwapnień w naczyniach oceniano w oparciu o wskaźnik uwapnienia naczyń wieńcowych (CaSc) i grubość kompleksu błony wewnętrznej i środkowej tętnicy szyjnej wspólnej (CCA-IMT). Gęstość mineralną kości mierzono przy użyciu aparatu Lunar DPX. Stężenie w surowicy rutynowo badanych wskaźników oceniano w aparacie Modular P analyzer (Roche Diagnostics), iPTH w oparciu o metodę Nicholsa, hsCRP i IL-6 przy użyciu techniki nefelometrycznej, podczas gdy fetuinę-A oznaczano w oparciu o zestawy ELISA.

Wyniki. W badaniu densytometrycznym wykazano obniżoną gęstość w zakresie kości udowej (Tscore = -2,0 ± 1,00) i odcinka lędźwiowego kręgosłupa (Tscore = -1,03 ± 1,51). Wskaźnik uwapnienia wskazywał na nasilone zwapnienia; CaSc – 511 (158-2394) jednostek Agatstona, a CCA-IMT – 0,90 ± 0,42. Stężenia fetuiny-A były istotnie ujemnie związane z wiekiem pacjentów (r = -0,26; p = 0,04), log (iPTH) (r = -0,31; p = 0,02), log (CRP) (r= -0.31, p = 0.02), log (IL-6) (r = -0.41, p = 0.001), log (CaSc) (r = -0,29; p = 0,03) i log (CCA-IMT) (r = -0,28; p = 0,04). Stężenia fetuiny-A dodatnio korelowały z albuminą (r = 0,37; p = 0,003) i BMD w okolicy szyjki kości udowej (r = 0,26; p = 0,04).

Wnioski. Pacjenci leczeni powtarzanymi hemodializami mają zaawansowane zwapnienia w układzie naczyniowym. Niskie stężenia fetuiny-A w surowicy są ujemnie związane ze wskaźnikami uwapnienia, zapaleniem i nadczynnością przytarczyc.

INTRODUCTION

Cardiovascular diseases are the leading cause of death in the population of patients with end stage renal disease on maintenance hemodialysis. The increased cardiovascular mortality in dialysis patients may be influenced by many factors, such as advanced age, atherosclerosis, chronic inflammation, malnutrition, hypertension, anemia, diabetes, left ventricular hypertrophy (LVH) and vascular calcification in the course of secondary hyperparathyroidism (1-3). Vascular calcifications may be observed even in the early decades of life in patients with end-stage renal disease (ESRD) and severity of calcification increases with the progression of renal failure (2-6).

Epidemiological studies have shown that severe vascular calcifications and associated increased risk of mortality occur particularly in patients with reduced serum fetuin-A levels (7-9). The fetuin-A acts as an inhibitor of extraosseous calcification (10-14), it inhibits the de novo formation of hydroxyapatite crystals, at least in part, causing the sequestration of calcium and phosphate, and preventing their precipitation in serum containing these minerals (12, 15). The fetuin-A accumulates in the vascular calcification (16) and its serum levels reduction in patients with ESRD is inversely correlated with the occurrence of calcification of the coronary arteries (17-20).

It was also found that in patients with ESRD calcified plaques were higher in those with low bone density (21). It was also demonstrated that reduced bone mineralization in patients with chronic kidney disease (CKD), or in post-menopausal women are accompanied with increased calcifications of tissues, especially vascular system (22-24).

AIM

The aim of the study was to assess the severity of calcifications in the cardiovascular system in patients treated with repeated hemodialysis depending on the concentrations of serum fetuin-A, bone mineral density and markers of inflammation.

MATERIAL AND METHODS

The study included 71 patients (31 women, 40 men) aged 60 ± 12 years on chronic dialysis because of end-stage renal failure for a period of approximately 75 ± 57.2 months. The cause of ESRD was chronic glomerulonephritis – 16 patients, chronic pyelonephritis – 16, kidney cirrhosis – 18, polycystic kidney disease – 10, diabetic nephropathy – 3 and not known cause of ESRD – 8. From the study group 55 patients had been treated for hypertension, and 8 had diabetes mellitus type 2. None of the patients had an active infections at the time of the study.

In 35 patients ischemic heart disease was diagnosed.

The basic demographic data and the results obtained are shown in table 1. Blood samples for routine laboratory tests were taken before hemodialysis start in the middle of the week sessions. The basic biochemical studies have been performed on the analyzer Modular P (Roche Diagnostic), C-reactive protein and IL-6 using immunonephelometric techniques Behring Nephelometer II, Dade Behring, Marburg, Germany while iPTH based on chemiluminescence Nichols method. The serum concentration of fetuin-A were measured using commercially available ELISA kits. Blood samples were centrifuged and the resulting serum/plasma aliquoted and stored at -70°C until immunoassays measurement. Routine laboratory tests were performed in the Diagnostic Laboratory at the University Hospital in Kraków, and immunochemical tests in Diagnostics Department, Jagiellonian University Medical College in Kraków.

The study was approved by the Jagiellonian University Bioethics Committee and all the patients gave written informed consent for participation.

Imaging techniques

The calcification score was measured by 64-slice spiral computer tomography scans, MSCT (Siemens Medical Solutions Inc., Germany) using the standard calcium scoring protocol. To quantify the calcification

Tab. 1. Demographic and clinical data of hemodialysis patients

Studied parameters	Whole group (N = 71)	Women (N = 31)	Men (N = 40)	p-value
Age (years)	60 ± 12	61 ± 14	59 ± 12	0.5
Dialysis vintage (months)	60 (36-100)	52 (30-108)	60 (45-97)	0.8
BMI (kg/m^2)	23.6 (21.0-26.7)	22.0 (20.0-25.5)	24.8 (22.6-28.0)	0.01*
Hypertension	55 (77%)	23 (74%)	32 (80%)	0.6
Diabetes mellitus type 2	8 (11%)	2 (6%)	6 (15%)	0.5
Ischemic heart disease	35 (49%)	19 (61%)	16 (40%)	0.08
Smoking	15 (21%)	5 (16%)	10 (25%)	0.4
BMD femoral neck (g/cm^2)	0.77 ± 0.17	0.73 ± 0.16	0.81 ± 0.17	0.07
BMD lumbar spine (g/cm^2)	1.08 ± 0.24	0.96 ± 0.20	1.17 ± 0.23	0.0004*
CaSc (Agatstone units)	488 (109-1853)	462 (41-1428)	511 (158-2394)	0.5
CCA-IMT (mm)	0.90 (0.80-1.05)	0.90 (0.75-1.00)	0.90 (0.80-1.05)	0.3

Values that were normally distributed, expressed as mean ± SD, data that were not normally distributed, expressed as median and (minimum-maximum), categorical variables are presented as %. *Significant associations
BM – body mass index; BMD – bone mineral density; CaSc – calcium scoring; CCA-IMT – common carotid artery intima-media thickness

of the coronary arteries indicator of coronary artery calcification was used, expressed in units of numerical values forming the sum of the Agatston score for final calcification of the coronary arteries (CaSc). Common carotid artery intima-media thickness (CCA-IMT) measurements were performed using a ALOKA 5500 SV equipped with a head for vascular studies. The CCA-IMT of the medial trunk-internal carotid artery was measured at three locations: at the height of half the length of the common carotid artery; at a distance of 1 cm from the bifurcation of the common carotid artery and at the height of the pad common carotid artery. In each of the three points a 2-3 medial thickness measurements during the diastolic phase of the cardiac cycle were performed, and the final results were calculated as an average arithmetic values at a point of measurement. Both, CaSc and CCA-IMT were performed at the Center for Diagnosis and Rehabilitation of Heart and Lung Diseases Specialist Pope John Paul II Hospital in Kraków. Bone mineral density (BMD) was performed in the Metabolic Diseases Clinic of the University Hospital in Kraków using Dual Energy X-ray Absorptiometry, Lunar DPX (Lunar, USA). BMD was measured in the lumbar spine (L1-L4) and femoral region (Ward's triangle).

Statistical analysis

The obtained data were reported as number (percent) and analyzed with Chi-squared test. Mean ± standard deviation (SD) or median (Lower-upper quartile) according distribution were given. Simple and multiple regression models were computed after log10-transformation of right-skewed variables.

The results were considered significant at $p \leq 0.05$.

RESULTS

The results of routine laboratory parameters, concentration of fetuin-A and imaging studies in the analyzed group of patients are summarized in table 1. Densitometry showed a decrease in bone mineral density both within the ranks of the femur (Tscore = -2.10 ± 1.00) and lumbar spine (Tscore = -1.03 ± 1.51). Calcium scoring performed in patients showed severe calcification of the vascular system, CaSc – 511 (158-2394) Agatston units and the CCA-IMT was 0.90 ± 0.42.

To evaluate the relationships of fetuin-A with selected variables the linear correlation tests were performed (tab. 2). In these tests fetuin-A concentrations were significantly negatively associated with patients age ($r = -0.26$, $p = 0.04$), log (iPTH) ($r = -0.31$, $p = 0.02$), (CRP) ($r = -0.31$, $p = 0.02$), log (IL-6) ($r = -0.41$, $p = 0.001$), log (CaSc) ($r = -0.29$, $p = 0.03$) and log (CCA-IMT) ($r = -0.28$, $p = 0.04$). Concentrations of fetuin-A were positively correlated with albumin ($r = 0.37$, $p = 0.003$) and BMD femoral neck ($r = 0.26$, $p = 0.04$).

Tab. 2. Selected laboratory values and correlation of fetuin-A with studied parameters (the Pearson`s correlation coefficient)

Parameters	Concentrations of laboratory parameters	Entire cohort	
		r	p
Age (years)		-0.26*	0.04*
Calcium (mmol/L)	2.30 ± 0.22	0.13	0.3
Phosphate (mmol/L)	1.87 ± 0.53	-0.24	0.07
Ca x Pi (mg^2/dl^2)	52.7 ± 15.11	-0.19	0.1
Log (iPTH) (pg/ml)	378 (132-1035)	-0.31*	0.02*
Albumin (g/L)	38.9 ± 3.4	0.37*	0.003*
Log (CRP) (mg/L)	5.71 (2.17-10.80)	-0.31*	0.02*
Log (IL-6) (pg/ml)	4.84 (0.70-7.25)	-0.41*	0.001*
BMD femoral neck (g/cm^2)		0.26*	0.04*
BMD lumbar spine (g/cm^2)		0.09	0.5
Log (CaSc) (Agatstone units)		-0.29*	0.03*
Log (CCA-IMT) (mm)		-0.28*	0.04*

Values that were normally distributed, expressed as mean ± SD, data that were not normally distributed, expressed as median and (minimum-maximum). Parameters that were not normally distributed were log10-transformed for analysis. *Significant associations
Ca x Pi – calcium phosphate product; iPTH – intact parathormone; CRP – C-reactive protein; IL-6 – interleukin-6; BMD – bone mineral density; CaSc – calcium scoring; CCA-IMT – common carotid artery intima-media thickness

In next step of our analysis we tent to investigate differences in relations between fetuin-A levels and analyzed parameters in women and men separately.

In the male group fetuin-A were significantly positively associated with levels of albumin (r = 51, p = 0.0008), negatively with levels of log (CRP) (r = -0.41, p = 0.008) and of IL-6 (r = -0.52, p = 0.0006). However no significant associations were observed between fetuin-A concentrations and the same inflammation parameters in female group (fig. 1). Additionally we observed that in male group the relationships between fetuin-A and inflammation markers are stronger than in whole patients group. When analyzed associations between fetuin-A and bone parameters depending on sex we showed that only in female group we observed statistically significant negative correlation between fetuin-A and log (iPTH) (r = -0.40, p = 0.03).

Relationships between fetuin-A and log (CaSc) remained only in male group, r = -0.36, p = 0.03 (fig. 2), and additionally between fetuin-A and log (CCA-IMT) lost their significance in both groups.

Correlation between fetuin-A levels and BMD of femoral neck almost reach statistical significance in men; r = 0.33, p = 0.06 (fig. 3).

Because CKD is accompanied by secondary hyperparathyroidism expressed by high levels of iPTH and chronic generalized inflammation we analyzed the extent to which correlate with serum fetuin-A. The study group of patients was divided into a group with serum iPTH $\leq$ 300 pg/ml and group with secondary hyperparathyroidism with iPTH > 300 pg/ml, and a group with low CRP

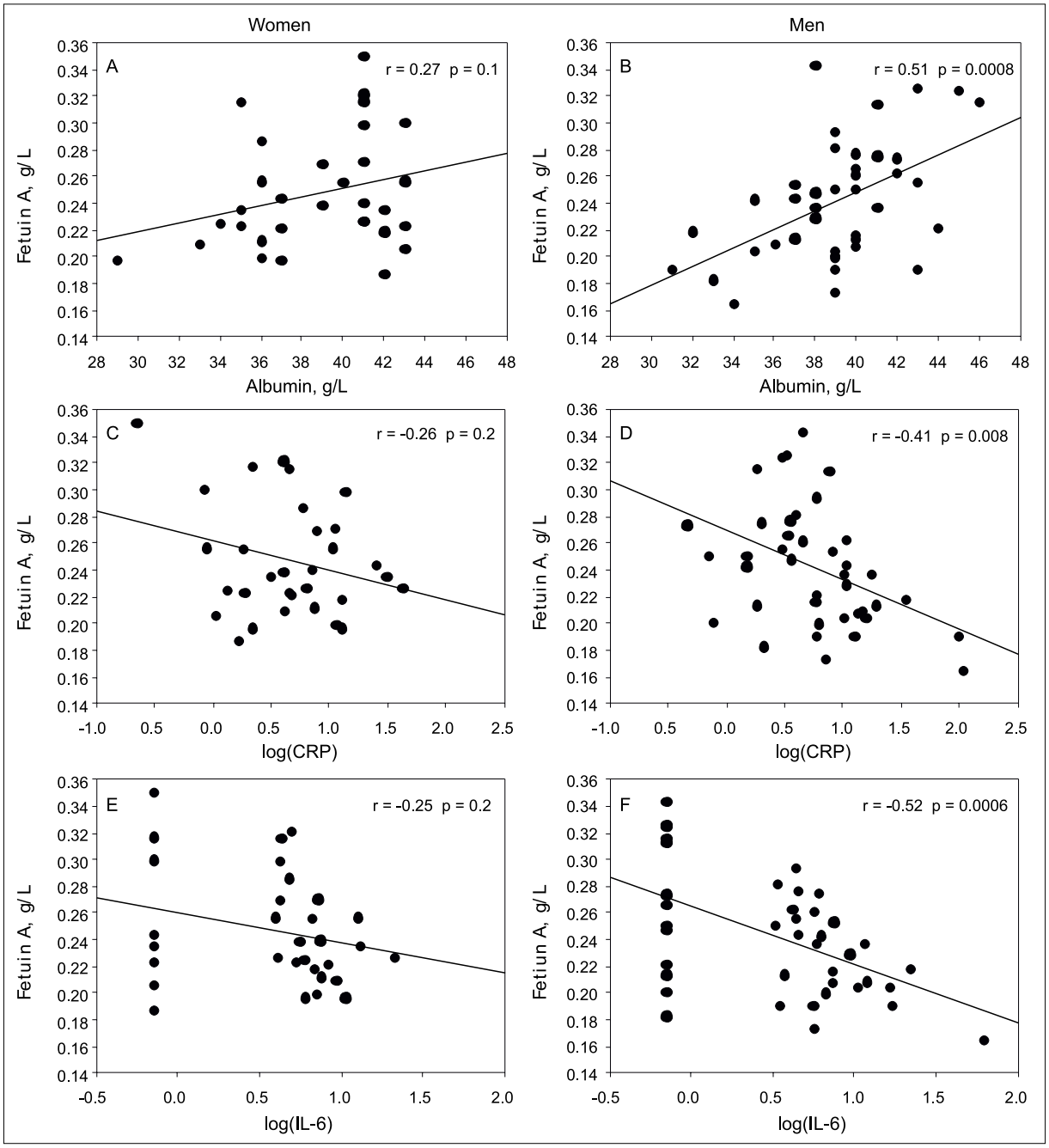

Fig. 1. Linear correlations of fetuin-A levels with albumin, log (CRP) and log (IL-6). A, C, E represents correlations in women. B, D, F represents correlations in men

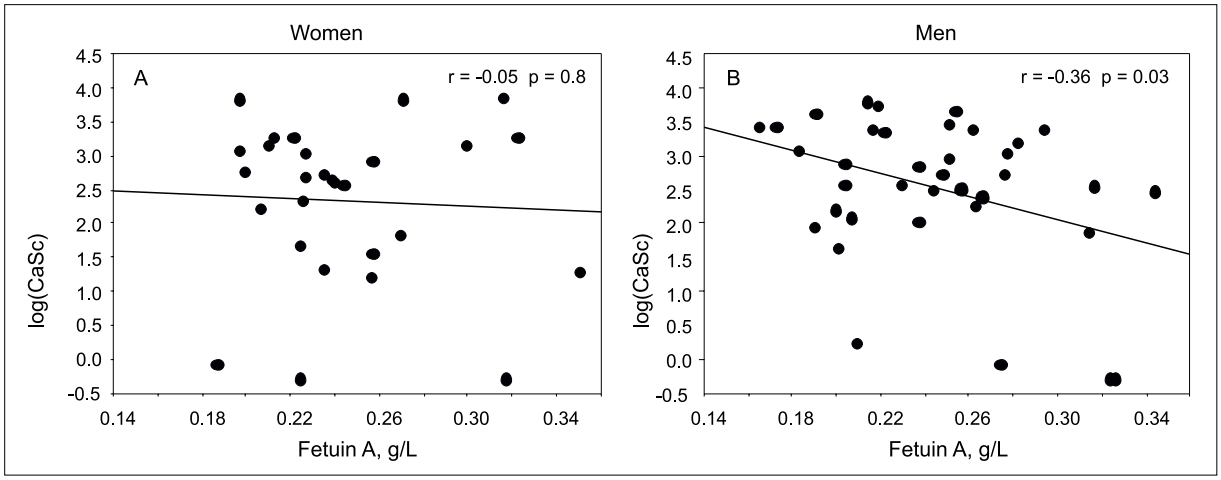

Fig. 2. Linear correlations of fetuin-A levels with log (CaSc) in women (A) and in men (B)

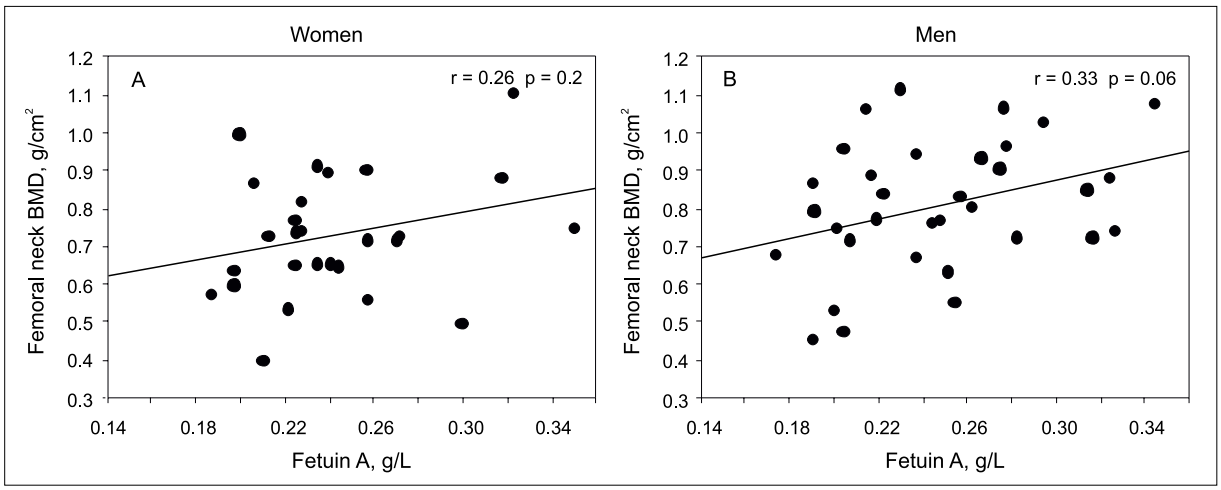

Fig. 3. Linear correlations of fetuin-A levels with femoral neck BMD in women (A) and in men (B)

≤ 10 mg/L and CRP > 10 mg/L (fig. 4). Both secondary hyperparathyroidism (p = 0.03) and inflammation (p = 0.01) were associated with decreased levels of fetuin-A in serum of patients on hemodialysis.

As the renal disease is accompanied by disorders of bone metabolism as a result of renal bone disease we tested using multiple regression model fetuin-A, CRP and PTH with bone mineral density. It has been shown that in the presence of PTH (model I), fetuin-A loses significance in relation to BMD, and when included in the analysis CRP (model II), fetuin-A remained an independent predictor of reduced bone mass in hemodialysis patients (tab. 3). Based on the multiple regression analysis for fetuin-A as the dependent variable has been shown that in patients on hemodialysis, CRP is independent of PTH predictor of reduced levels of fetuin-A (tab. 4).

Tab. 3. Multiple regression models for femoral neck BMD as the dependent variable

The explanatory variables	Model I		Model II	
	beta	p	beta	p
Fetuin-A	0.20	0.1	0.32*	0.02*
log (CRP)	–	–	0.05	0.7
log (iPTH)	-0.28*	0.03*	–	–

*Statistically significant

Tab. 4. Multiple regression models for fetuin-A as the dependent variable

The explanatory variables	Model III		Model IV	
	beta	p	beta	p
log (CRP)	-0.29*	0.01*	-0.29*	0.01*
log (iPTH)	-0.22	0.06	-0.13	0.4

*Statistically significant

DISCUSSION

The main findings of this cross-sectional study performed in in the group of ESRD patients on maintenance hemodialysis are as follows:

1. Diminished serum levels of fetuin-A in hemodialysed patients was both associated with the extent of CaSc and CCA-IMT.
2. The serum fetuin-A levels were negatively associated both with inflammation expressed as increased serum hsCRP levels and IL-6 as well as secondary hyperparathyroidism with high iPTH serum levels.
3. Fetuin-A levels showed negative correlation with inflammatory parameters only in men, but in women it correlates only with log (iPTH).
4. The serum fetuin-A levels correlated positively with BMD of the femoral neck but not BMD of

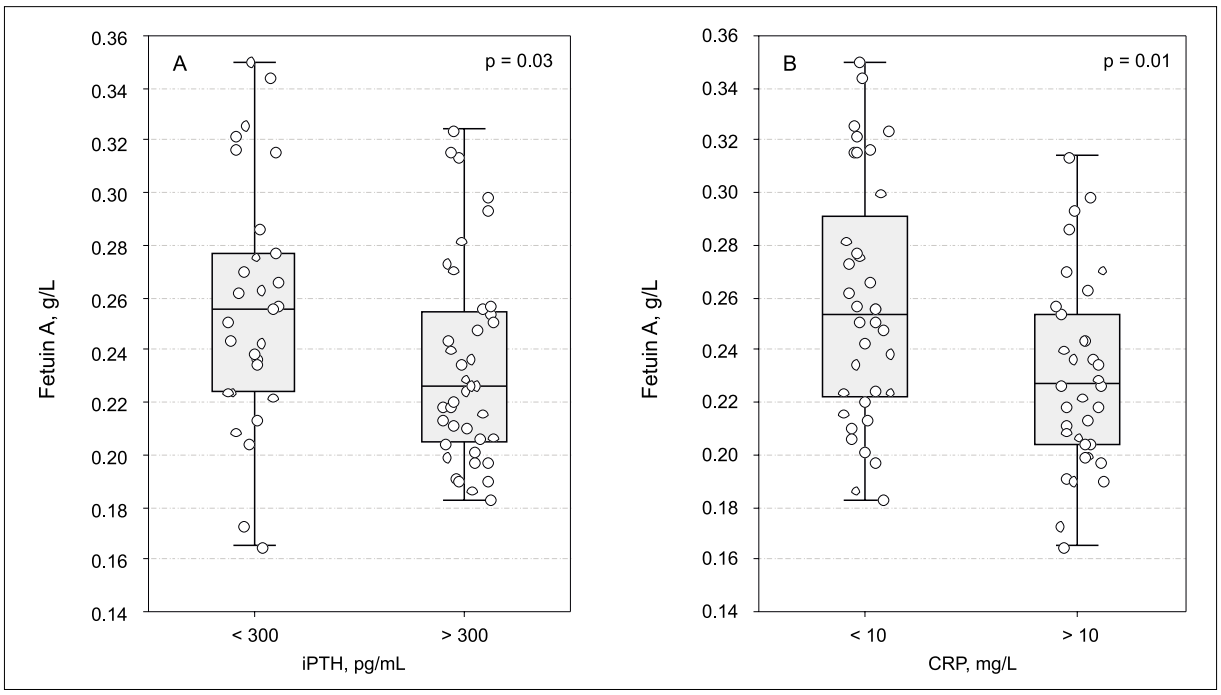

Fig. 4. (A) Serum fetuin-A concentration in relation to secondary hyperparathyroidism, defined as concentrations iPTH > 300 pg/ml. (B) Serum fetuin-A concentration in relation to inflammation, defined as concentrations CRP > 10 mg/L. Data are shown as median, interquartile range (box) and minimum-maximum (whisker). Open circles represent row data

lumbar spine, and in relation to the femoral neck BMD low fetuin-A was dependent of PTH, but independent of CRP associated with decreased bone mass.

In our study we found that reduced levels of fetuin-A correlated with the severity of coronary artery calcification expressed as CaSc and CCA-IMT.

Cardiovascular disease is the most common cause of mortality in hemodialysis patients, in whom cardiovascular mortality increased 10-20 times compared to the general population (25). High values of coronary artery calcifications expressed as CaSc obtained in patients on hemodialysis, and thickening of the intima-media layers, can indicate the presence of a high risk of cardiovascular morbidity in the study group. At the same time there was decrease in bone mineral density, which in ESRD patients is an expression of renal osteodystrophy.

Coronary calcifications documented in patients with ESRD patients, the severity of the atherosclerotic vascular diseases are predictors of cardiovascular events (2, 3). Both the changes observed in clinical imaging of arteries, hyperphosphatemia and bone metabolism disturbances as a results of secondary hyperparathyroidism in conjunction with chronic inflammatory process, are the risk factors for increased morbidity and cardiovascular mortality in this group of patients (26, 27).

Although, vascular calcification occurs in most patients with CKD, some patients do not develop calcification, despite exposure to the uremic environment. Physiological calcification inhibitors, among which an important is fetuin-A, may play a role in inhibiting the formation and progression of metastatic calcification but the results of clinical trials are few and often contradictory. Several researchers have observed a relationship between low levels of fetuin-A and vascular calcification (12, 19, 28-30), although others found no similar relationship (31, 32), as there was no relationship between levels of fetuin-A and CaSc in predialysis patients (33). Vascular calcification leads to stiffening of the arteries. Fetuin-A endogenous inhibitor of vascular calcification is associated with arterial stiffness and mortality in dialysis patients without diabetes (34) and in patients in the predialysis stage (35). In contrast, Hermans et al. (32) showed no differences between serum fetuin-A of dialysis group and normal healthy control subjects, and showed no relationship between serum fetuin-A and arterial stiffness. In the general population findings are inconsistent, because fetuin-A levels were positively associated with CaSc in patients not undergoing dialysis with diabetic nephropathy (31). Based on epidemiological studies it was found that fetuin-A acts as an inhibitor of systemic ectopic calcification (14), but the clinical significance of the concentration of fetuin-A is not entirely clear. Although a multicenter prospective study involving patients with HD and PD showed that the concentration of fetuin-A may be a predictor of overall mortality in dialysis patients (36, 37). Similarly it was shown a significant association between reduced levels of fetuin-A and calcification of heart valve, atherosclerosis, malnutrition and inflammation and has been associated with mortality and cardiovascular events in PD patients (9, 29, 38, 39).

The relationship between serum fetuin-A levels and BMD has already been confirmed, particularly

in hemodialysis patients in whom renal osteodystrophy coexists with vascular calcification (40-44). Numerous studies indicate a high similarity of the pathophysiological mechanisms responsible for the progression of vascular calcification plaque to the process of bone formation. It was also found that higher levels of fetuin-A are significantly associated with higher BMD total hip, lumbar spine, and whole body bone mineral density among well functioning community-dwelling older women (45). Calcification and plaque volume were higher in patients whose T-score < -2.5 in the region of proximal radius, neck and trochanter of the femur and lumbar spine. CaSc, weight and volume of plaque in the coronary arteries were significantly correlated with serum fetuin-A, BMD and T-score proximal radius, neck, trochanter of the femur, but not with the lumbar spine. It was also shown a relationship between serum fetuin A levels, CaSc, CCA-IMT, cardiovascular events and BMD in chronic hemodialysis patients (21, 42, 46, 47). In patients with ESRD on HD apparently rate of bone turnover depends largely on the concentration of circulating parathyroid hormone and it is a major determinant of BMD in this patients, and the relationship between serum fetuin-A levels and BMD is associated with hyperparathyroidism (48, 49). Renal osteodystrophy accompanying CKD is associated with an increase in PTH, CRP and reduction of bone mineral density (7, 30, 43, 49).

It has been shown in our study that the reduced concentrations of fetuin-A is accompanied with reduced bone mass – BMD of femoral neck. In addition, we examined the relationship between BMD and fetuin-A in a model of multiple regression analysis. In this model it was shown that fetuin-A in the presence of PTH loses significance in relation to BMD, however in the presence of CRP (model II), fetuin-A remained an independent predictor of reduced bone mass in hemodialysis patients. The results may indicate the role of fetuin-A in maintaining normal bone mass, and that her relationship with bone mass depends on the secondary hyperparathyroidismus, and perhaps accelerated bone turnover in these patients. These observations was confirmed by the multiple regression determinants analysis of fetuin-A in serum of patients on hemodialysis. It has been shown that independent predictor of the concentrations of fetuin-A, CRP, and skeletal parameters exert their effect on fetuin-A in a manner dependent from each other. So accelerated bone turnover, caused an increase in PTH and an increased release of bone markers is accompanied by lowering levels of fetuin-A levels in hemodialysis patients. When studied group of patients were divided into subgroups according to the iPTH and CRP we showed that in patients undergoing hemodialysis, both chronic inflammation and secondary hyperparathyroidism are associated with decreased levels of fetuin-A.

In our study we observed lack association of fetuin-A with CaSc, and BMD femoral neck in women. It is possible that in women dependent of sex factors had an influence on relation of fetuin-A, to other variables. It was shown in other study that the concentrations of fetuin A did not differ between men and women (50). Fetuin-A is the major bone non non-collagenous protein. *In vitro* fetuin A can inhibit or stimulate osteogenesis, depending on its concentration (51). Association of the concentrations of fetuin-A with bone density and markers of bone resorption (52) in healthy women without diabetes, but not in men (45) suggests the possibility of a sexually dimorphic contribution of fetuin-A to the development of osteoporosis and bone fractures, and probably also there is sexual dimorphism in relation fetuin-A and vascular calcification. The presence of low bone mineralization, for example in CKD patients, or post-menopausal women are associated with the ectopic calcification, particularly vascular wall (22, 23). It is well documented that estrogen decreases after menopause in women. Estrogens deficiency results in a sharp increase in bone turnover markers (53). Previous studies have shown a link between levels of fetuin-A and BMD in healthy older women but not in men (45). Although the mechanism underlying sexual dimorphism according to fetuin-A and biomarkers of bone turnover and vascular calcification is still unclear, there may be sex-dependent influence of fetuin-A on activity of osteoclasts and osteoblasts, or as a secondary effect of sex hormones on fetuin-A. Fetuin-A is positively associated with estradiol level in women, therefore it is not excluded that fetuin-A promotes the mineralization of bone in post-menopausal women with osteoporosis, which is characterized by low levels of estrogen (50).

The postmenopausal women with the most rapid bone loss (and therefore the greatest imbalance between resorption and formation within the bone remodeling compartment) have the most rapid rate of artery calcifications (54-57). For the above reasons fetuin-A is negatively correlated with inflammation parameters only in men, but in women it correlates with log (iPTH), but not with inflammation.

CONCLUSIONS

1. Patients on maintenance hemodialysis suffer from severe calcifications of vascular system.
2. Low serum fetuin-A levels are negatively associated with calcification parameters, inflammation and hyperparathyroidism.

Limitations

The result of the present work should be interpreted with caution because of several limitations. First, the sample size was small. Second, the cross-sectional nature of the study may also influence the results. Third, fetuin-A levels were only assessed at single point in time instead of having time-averaged values and these values are related to bone mineral densitometry parameters and coronary artery calcification that develop

over a long time. In addition, the concentration of fetuin-A may fluctuate, largely depending mainly on the status of inflammation and iPTH (21).

Lack of standardization of fetuin-A assays causes confusion and discordancy (58). The fetuin-A is an important inhibitor of extraosseous calcification, but in some studies using ELISA assays showed no significant correlation between serum fetuin-A and vascular calcification in patients with CKD (59). This mismatch results is explained by several reasons. One reason may be the lack of harmonization of methods for the determination of fetuin-A. A commercially available various methods for the determination of the protein generate different values (58). Another reason for this situation may be the ability of fetuin-A to form the microparticles, protein and minerals that depending on the preparation used for centrifugation of blood which can affect the obtained results.

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