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Valve calcification and coronary artery calcification in peritoneal dialysis patients

Kalcyfikacja zastawek i naczyń wieńcowych w dializie otrzewnowej

In the course of chronic kidney insufficiency due to renal impairment almost more than 50% of deaths among patients with end-stage renal disease are due to cardiovascular diseases [6]. The risk of death is higher in hemodialysed than general population. Increased mortality rate as a result of damaged cardiac tissues after calcification may lead to abnormal conduction and arrhythmia, left ventricular dysfunction, aortic and mitral stenosis and regurgitation, complete heart block, ischemia, congestive cardiac failure and death [7].

MATERIAL AND METHODS

The study was performed in a group of peritoneal dialysis (PD) patients treated at the Nephrology Department of Medical University of Lublin. We examined a group of 45 patients maintained PD aged 26–82 years (mean 49.6). There were 25 women at the age 26 to 82 (mean age 49.4 years) and 20 men at the age 22 to 75 (mean age 49.8 years). Patients were being peritoneal dialyzed with two standards. At the first standard 25 patients were treated using continual peritoneal dialysis (CAPD) and the remaining 20 patients were treated using peritoneal automatic dialysis (APD). There were 14 women and 11 men (mean age 48. 9 years) in group ADO. Duration of dialyzotherapy in patients CDO was mean 48 ± 27 months and in group ADO was mean 52 ± 32 months. Samples of venous blood were collected to dry tubes without anticoagulant and they were centrifuged for 10 minutes. After serum separation, analyses were preformed. A part of serum was frozen at - 20˚C for fetuin – A analysis. Serum fetuin – A was determined using a human fetuin – A enzyme linked immunosorbent assay (ELISA) kit (Bio Vendor Laboratory Medicine, Inc.). Concentrations of C-reactive protein, calcium, phosphorus, lipid profile (total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglyceride), fibrinogen were measured using standard methods on the HITACHI 902 Roche Diagnostic biochemistry analyzer and Ca x P product was calculated.

Parathyroid hormone (PTH) was determined by chemiluminescence immunoassay on the Immulite 1000 analyzer (Siemens Medical Solutions). Calcification of coronary vessels was estimated using Spiral Computer Tomography at the Department Radiology of Medical University of Lublin.

RESULTS

The obtained results were analyzed with the use of Statistica software. All values are expressed as median and standard deviations. The distribution of variables was analyzed using Shapiro-Walk's. The correlation analyses of the variables were carried out using the Spearman test. Nonparametric statistical analysis was performed using U Mann-Whitney's test. P value < 0.05 was considered to be statistically significant.

The studies were preformed in three steps. Firstly, patient were grouped according to Agatson score. Group I, those without coronary vessels calcification (Agatson score = 0); group II, those with mean coronary vessels calcification (Agatson score < 400); group III, those with coronary vessels calcification (Agatson score >400). In the three groups we evaluated the proportion of patients with VC. We did not find patients without VC in the group. There was no presence of cardiac valve calcification. In the group with mean coronary vessels calcification, we noticed 32 % VC and 85% was in group III. The differences were significant ($p < 0.05$).

The studies evaluated the frequency of occurrence of mitral valve calcification (Table 1), as well as aortic valve calcification (Table 2) in group with valve calcification. Moreover, in the examined group we observed mitral valve calcification in tomography. Of these patients, three had mitral valve calcification, 16 patients had aortic valve calcification and 2 patients appeared to have both mitral valve calcification and aortic valve calcification. After taking into consideration the patient's sex, we were unable to show a correlation of the frequency of occurrence of coronary vessels calcification and valve calcification in the groups of women and men. We did not find significant differences in the evaluated parameters depending on the patients' sex . In the group of peritoneal dialysis we showed significant relations of the patients' age with coronary vessels calcification in comparison with patients without valvular calcification. Furthermore, we found fundamentally higher values of Agatson score and volumetric units in comparison to patients without calcification ($p<0.05$).

Table 1. Patients number with Mitral Valve Calcification

Coronary Artery Calcification	Mitral Valve Calcification		
	Lack	Present	Sum
Lack	13	0	13
% Verse	100.00%	0.00%	
Mean	18	1	19
% Verse	94.74%	5.26%	
Significant	11	2	13
% Verse	84.62%	15.38%	
Sum	42	3	45

NS

Table 2. Patients number with Aortic Valve Calcification

Coronary Artery Calcification	Aortic Valve Calcification		
	Lack	Present	Sum
Lack	13	0	13
% Verse	100.00%	0.00%	
Mean	13	6	19
% Verse	68.42%	31.58%	
Significant	3	10	13
%Verse	23.08%	76.92%	
Sum	29	16	45

$p < 0.001$

Table 3. Biochemical parameters for the groups with and without valve calcification

Valve calcification	Absent	Present	p
Age (years)	41.4 ± 12.40 *(40.00)*	63.0 ± 13.17 *(64.00)*	p < 0.001
CRP (mg/l)	16.68 ± 24.67 *(10.73)*	32.52 ± 39.91 *(23.00)*	p < 0.05
Fibrinogen (g/l)	5.26 ± 1.36 *(5.33)*	6.99 ± 1.09 *(6.87)*	p < 0.001
Calcium (mg/dl)	9.12 ± 0.80 *(9.2)*	9.01 ± 0.84 *(8.9)*	NS
Phosphate (mg/dl)	5.05 ± 1.94 *(4.57)*	5.39 ± 1.72 *(5.60)*	NS
Ca x P product (mg^2/dl^2)	45.91 ± 18.42 *(40.83)*	48.11 ± 14.38 *(47.90)*	NS
Total cholesterol (mg/dl)	246.89 ± 55.84 *(225.50)*	233.76 ± 33.09 *(238.0)*	NS
HDL (mg/dl)	51.14 ± 15.54 *(49.0)*	46.58 ± 32.0 *(46.0)*	NS
Triglyceride (mg/dl)	193.57 ± 79.18 *(168.0)*	204.11 ± 138.71 *(169.0)*	NS
LDL (mg/dl)	157.03 ± 47.61 *(140.9)*	146.35 ± 30.13 *(146.4)*	NS
Homocysteine (mmol/l)	17.27 ± 5.79 *(16.78)*	20.21 ± 9.18 *(19.06)*	NS
PTH (ng/l)	667.54 ± 642.52 *(411.0)*	397.30 ± 389.30 *(289.0)*	NS
Fetuin A (ng/ml)	20.3 ± 2.6 *(20.2)*	17.7 ± 3.3 *(17.5)*	< 0.05
Agatson score	156.8 ± 382.7 *(5.0)*	1125.5 ± 1115.6 *(731.0)*	< 0.001
Volumetric units	140.9 ± 305.8 *(8.5)*	988,9 ± 913,1 *(637.0)*	< 0.001

Table 3 shows the results in the group with and without cardiac valve calcifications. According to the correlation analysis of C-reactive protein, fibrinogen, fetuin – A seems to be a more valuable marker in calcification. Evaluation was made of the marked parameters between Agatson score, volumetric units at a later stage. All the patients with coronary vessels calcification showed a statistically significant positive association between age and Agatson score, volumetric score. Several studies noted a positive correlation between values of calcification in Agatson score, volumetric units and concentrations of C-reactive protein, fibrinogen. There were also, in the group without cardiac valve

Table 4. Correlation between Agatson and volumetric units with chosen parameters for the groups with and without valve calcification

	R	P
Agatson score & age	0.482	< 0.05
Agatson score & CRP	-0.053	NS
Agatson score & fibrinogen	0.487	< 0.05
Agatson score & Ca	0.191	NS
Agatson score & P	-0.367	NS
Agatson score & Ca x P	-0.267	NS
Agatson score & TC	-0.089	NS
Agatson score & HDL	0.221	NS
Agatson score & TG	0.060	NS
Agatson score & LDL	-0.325	NS
Agatson score & HCY	0.028	NS
Agatson score & PTH	-0.415	NS
Agatson units & Fetuin A	-0.425	< 0.05
Volumetric units & age	0.445	< 0.05
Volumetric units & CRP	-0.075	NS
Volumetric units & fibrinogen	0.519	< 0.05
Volumetric units & Ca	0.243	NS
Volumetric units & P	-0.385	NS
Volumetric units & Ca x P	-0.260	NS
Volumetric units & TC	-0.071	NS
Volumetric units & HDL	0.269	NS
Volumetric units & TG	0.107	NS
Volumetric units & LDL	-0.328	NS
Volumetric units & HCY	0.021	NS
Volumetric units & PTH	-0.432	NS
Volumetric units & Fetuin-A	-0.478	< 0.05

calcifications (Table 4), present essential correlations between values indices of calcifications and age of the examined group, fibrinogen, fetuin – A ($p<0.05$). Age is a risk factor for cardiac VC. In the group of patients with valve calcifications we showed a statistically significant positive correlation between indices of calcifications and concentrations of C-reactive protein (Table 5).

DISCUSSION

Renal insufficiency is a long term progressive disease leading to the patient`s death. An efficient method of treatment is dialysotherapy, which may maintain incurably ill patient alive but only successful renal transplantation may be a rescue from death. Patients with chronic kidney disease,

Table 5. Correlation between Agatson and volumetric units with chosen parameters for the groups with valve calcification

	R	P
Agatson score & age	0.490	< 0.05
Agatson score & CRP	0.571	< 0.05
Agatson score & fibrinogen	0.603	< 0.05
Agatson score & Ca	0.196	NS
Agatson score & P	-0.296	NS
Agatson score & Ca x P	-0.196	NS
Agatson score & TC	0.356	NS
Agatson score & HDL	0.098	NS
Agatson score & TG	0.253	NS
Agatson score & LDL	0.101	NS
Agatson score & HCY	-0.014	NS
Agatson score & PTH	-0.002	NS
Agatson score & Fetuin A	0.658	< 0.01
Volumetric units & age	0.435	< 0.05
Volumetric units & CRP	0.598	< 0.05
Volumetric units & fibrinogen	0.590	< 0.05
Volumetric units & Ca	0.128	NS
Volumetric units & P	-0.259	NS
Volumetric units & Ca x P	-0.203	NS
Volumetric units & TC	0.337	NS
Volumetric units & HDL	0.135	NS
Volumetric units & TG	0.211	NS
Volumetric units & LDL	0.111	NS
Volumetric units & HCY	-0.022	NS
Volumetric units & PTH	0.026	NS
Volumetric units & Fetuin-A	0.646	< 0.01

especially the hemodialysis patients, frequently develop widespread cardiac and vascular calcification [3]. Cardiac disease is the major cause of death among end-stage renal disease (ESRD) patients, accounting for nearly 50% of all deaths in this patient population [6]. It is generally accepted that ESRD patients are at greater risk for myocardial ischemia and infraction. Indeed, the death rate attributed to these conditions is at least several-fold higher for ESRD patients as compared to the general population, likely due to both classic risk factors, such as hypertension and lipid abnormalities, and to risk factors unique to ESRD [13]. Vascular calcification mechanism is not fully understood. But we known that VC is a dynamic process in which the vessel wall intima, media and also cardiac valves may be involved [1]. Intimal calcification is an endochondral ossification process in which type II collagen is mineralized by calcium deposition [1,14]. In contract, an intra-membranous ossification process leads to medial calcification, while a dystrophic calcification process is responsible

for valvular calcification [1, 3]. Mechanisms involved in VC may be summarized as: 1. Activation of oesteogenesis in the vessel wall, 2. Loss of inhibitory factors, 3. Enhanced bone turnover, and 4. Abnormalities in mineral metabolism.

In this study, we have demonstrated that valvular calcification (VC) is a common finding in end-stage renal disease (ESRD) patient undergoing peritoneal dialysis [18]. Development of atherosclerosis and its cardiovascular complications are observed in the accelerated from in this group of patients; therefore, mortality caused by cardiovascular disease is 70% in the group of peritoneal dialysis compared to patients with proper renal function. Chronic uremia is associated with numerous dysfunction affecting multiple organ systems [18]. Among complications of dialysotherapy mentioned in literature for patients with CKD disturbances there are protein carbohydrate, lipids metabolism, water-electrolyte balance, endocrine disorders, acid-base balance disturbances unspecific bacterial infections and arterial hypertension, atherosclerosis that are characteristic [13]. In agreement the with data from medical literature, we found association between fibrinogen, CRP as a biological marker of inflammation and calcification mechanism [18, 19]. In accordance with medical literature, Sharma et al. [13] reported that high sensitivity C-reactive protein was higher in patients with mitral valvular calcification (MVC) than those without, but the difference was not significant. Tourn et al.[15] also demonstrated that the patients with VC had significantly higher fibrinogen and CRP levels. In agreement with these findings, we found that MVC had higher serum levels of CRP than the group without VC.

In addition, an elevated level of CRP is associated with mitral valve calcification. In this study, we have demonstrated strong correlation between age and intensity of calcification which was measured by computer-assisted tomography (CAT). In agreement with the data from medical literature, Urena et al., in a multivariate analysis, found an association between aortic stenosis and increased age [16]. Ribeiro et al. reported that mitral VC was associated with age and higher Ca x P, and VC was related to age and duration of hypertension [11, 12]. Floege reported that age and duration of dialysis influence calcification mechanism and the older patient, the more likelihood of VC [2]. Previous studies reported that cardiac valve calcification is mainly characteristic of older patients (mean age 63.0 ± 13.2).

Fetuin-A is synthesized by hepatocytes and presents as a major soluble inhibitor of calcification in the extracellular space [17, 19]. Targeted deletion of the fetuin gene in calcification-sensitive mice has been associated with severe calcification in various organs, suggesting that fetuin-A is an important inhibitor of ectopic calcification acting on the systemic level. Price et al. showed that a specific complex of the calcium, phosphate, fetuin-A and matrix Gla. protein in the serum prevents bone mineralization in rats [9, 10, 13]. A recent study by Kettler and co-workers demonstrated an impaired capacity of serum from AHSG-deficient patient on long-term dialysis with clinical evidence of extra-osseous calcifications to inhibit calcium x phosphorus precipitation ex vivo [4, 5]. However, we found a negative correlation between the concentration of fetuin-A between volumetric units and Agatson score, which indicates that it is deficiency of fetuin-A lying at the fundaments of this pathology. This gave additional evidence that serum fetuin-A may indeed be an important inhibitor of calcification in dialysis patient.

There are many possible explanations for nephrotic syndrome. Nephrotic syndrome may occur when the filtering units of the kidney are damaged. The clinical picture characteristic of lipids

disorders includes increased total cholesterol, low density lipoprotein, triglycerides and decreased high density lipoprotein which tends to increase the risk of heart diseases and atherosclerosis [8, 14]. Our study showed no association between the lipid profile in the patient with or without VC. In our investigation, we did not conclude any correlation between the indices of calcification and lipid profile.

CONCLUSIONS

Calcification complications are frequent among peritoneal dialysis patients. In this study, we found that coronary vessels calcification is a common finding in 70% group of peritoneal dialysis. However, we found a negative correlation in the concentration of fetuin-A between volumetric units and Agatson score, which indicates deficiency of fetuin-A is fundamental in this pathology. VC is mainly characteristic of patients with high indices of coronary vessels calcification. In our study, patients with valvular calcification (VC) had significantly lower concentrations of fetuin-A than patients without VC. We found an important association between fibrinogen, CRP as a biological marker of inflammation and calcification mechanism. This factors and fetuin-A, being so involved in this process, can be a predictive factors for the evaluation of the risk of calcification above osteal and also atherosclerosis. In addition, the elevated level of CRP is associated with mitral valve calcification. Our results indicate that valvular calcification may be useful in stratifying the severity of atherosclerotic vascular disease in peritoneal dialysis patients. In summary, our study shows an important association between inflammation, CRP, fibrinogen and serum fetuin-A in peritoneal dialysis patients.

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SUMMARY

In the course of chronic kidney insufficiency due to renal impairment almost more than 50% of deaths among patients with end - stage renal disease are due to cardiovascular diseases [6]. The risk of death was higher in hemodialysed than general population. Increased mortality rate as a result of damaged cardiac tissues after calcification may lead to abnormal conduction and arrhythmia, left ventricular dysfunction, aortic and mitral stenosis and regurgitation, complete heart block, ischemia, congestive cardiac failure and death [7]. Our aim was to determine the prevalence of valvular calcification and coronary artery calcification in peritoneal dialysis patients and to examine some possible etiologic factors for its occurrence. Calcification complications are frequent among peritoneal dialysis patients. In this study, we have found that coronary vessels calcification is a common finding in 70% group of peritoneal dialysis. However, we found a negative correlation in the concentration of fetuin-A between volumetric units and Agatson score which indicates that it is deficiency of fetuin-A, fundamental of this pathology. VC is mainly characteristic of patients with high indices of coronary vessels calcification. In our study, patients with valvular calcification (VC) had significantly lower concentrations of fetuin-A than patients without VC. We found an important association between fibrinogen, CRP as a biological marker of inflammation and calcification

mechanism. This factors and fetuin-A, being so involved in this process, can be predictive factors for evaluation of risk of calcification above osteal and also atherosclerosis. In addition, the elevated level of CRP is associated with mitral valve calcification. Our results indicate that valvular calcification may be useful in stratifying the severity of atherosclerotic vascular disease in peritoneal dialysis patients. In summary, our study shows important association between, inflammation: CRP, fibrinogen and serum fetuin-A in peritoneal dialysis patients.

STRESZCZENIE

Pacjenci z przewlekłą chorobą nerek leczeni dializami stanowią populację wysokiego ryzyka przyspieszonego rozwoju miażdżycy, a około 50% śmiertelności w tej grupie chorych występuje z powodu chorób sercowo-naczyniowego. Ryzyko śmierci wynikające z tych przyczyn jest 20-30 krotnie wyższe niż w populacji ogólnej. Występowanie wysokich wartości wskaźnika śmiertelności na skutek niedokrwiennej choroby serca związane jest z obecnością nasilonej kalcyfikacji środkowej błony ściany aorty, co przyczynia się do wzrostu sztywności ścian tętnic, zwiększenia wartości ciśnienia tętna oraz spadku perfuzji przez tętnice wieńcowe podczas okresu rozkurczu, jak też kalcyfikacji zastawek serca. Celem pracy była ocena wzajemnych zależności pomiędzy stężeniami wybranych parametrów biochemicznych a stopniem kalcyfikacji zastawek i naczyń wieńcowych u pacjentów z przewlekłą niewydolnością nerek leczonych dializami otrzewnowymi Kalcyfikacja naczyń wieńcowych i zastawek serca występuje u ponad 70% pacjentów z przewlekłą niewydolnością nerek leczonych dializą otrzewnową. Stężenia endogennego inhibitora procesu kalcyfikacji – fetuiny-A – korelują w sposób ujemny z wartościami wskaźników kalcyfikacji naczyń wieńcowych (wskaźnik Agatsona i wolumetryczny), co wskazuje na to, że niedobór fetuiny-A leży u podstaw tej patologii. Kalcyfikacja zastawek serca jest charakterystyczna głównie u pacjentów, u których stwierdza się wysokie wartości wskaźników kalcyfikacji naczyń wieńcowych. U pacjentów z kalcyfikacją zastawek stężenia fetuiny-A są istotnie niższe w porównaniu z osobami bez zwapnień w obrębie zastawek. Wraz z nasileniem procesu kalcyfikacji naczyń wieńcowych i zastawek dochodzi do aktywacji reakcji ostrej fazy, co wyraża się wzrostem stężenia białka C-reaktywnego i fibrynogenu. Parametry te wraz z oznaczeniami fetuiny-A mogą być pomocnym narzędziem w ocenie ryzyka kalcyfikacji pozakostnej jak też miażdżycy.