Relations between serum sex hormone levels and biomarkers of atherosclerosis and mineral disturbances in postmenopausal chronic haemodialysis women

Zależności pomiędzy stężeniem w surowicy hormonów płciowych i biomarkerów zmian miażdżycowych a zaburzeniami gospodarki mineralnej u przewlekle hemodializowanych kobiet w okresie pomenopauzalnym

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Summary

Objectives: Both cardiovascular calcification and atherosclerosis are strong predictors of cardiovascular events in patients with end-stage renal disease. The aim of our study was to assess the presence and interrelations of sex hormone profile with coronary artery calcification (CAC), atherosclerotic plaques (AP) in the carotid artery and common carotid artery intima media thickness (CCA-IMT) in postmenopausal chronic haemodialysis (HD) women.

Material and methods: CCA-IMT and presence and thickness of AP were measured with high-resolution ultrasound and CAC with multidetector computed tomography in a cross-sectional study of 22 postmenopausal HD women without any history of major cardiovascular complications. Serum mineral parameters, lipids, estradiol, progesterone and testosterone were also measured.

Results: The CAC was detected in 72% of examined women. Mean CAC score in HD women was 770 ±1065 Agatston units. Mean thickness of CCA-IMT was 0.94 ±0.23 mm. The women who did not develop vascular calcification had lower CCA-IMT. In women without CAC the serum level of estradiol was significantly lower than in those with detectable CAC (28.2 ±8.2 *vs* 61.5 ±18.4 pg/mL). There was a tendency for higher serum estradiol in HD women with atherosclerotic lesions in the common carotid artery. Strong correlations between the serum level of estradiol and, respectively CAC score, CCA-IMT and AP were observed. We did not find any significant differences between anthropometric parameters, other laboratory parameters, progesterone, testosterone and the presence of cardiovascular complications.

Conclusions: The study results do not support the concept of cardiovascular protective effects of endogenous estrogens in postmenopausal chronic haemodialysis women.

Key words: estrogens, menopause, haemodialysis, cardiovascular complications.

Streszczenie

Wstęp: Zwapnienia naczyniowe oraz zmiany miażdżycowe stanowią czynniki ryzyka powikłań sercowo-naczyniowych u chorych ze schyłkową niewydolnością nerek. Celem pracy była ocena obecności i wzajemnych zależności pomiędzy stężeniem hormonów płciowych w surowicy a zwapnieniami w tętnicach wieńcowych (CAC), grubością kompleksu *intima-media* tętnicy szyjnej wspólnej (IMT) oraz obecnością i grubością blaszki miażdżycowej w tętnicy szyjnej wspólnej (AP) u przewlekle hemodializowanych kobiet w okresie pomenopauzalnym.

Materiał i metody: W przekrojowym badaniu, do którego zakwalifikowano 22 przewlekle hemodializowane kobiety w okresie pomenopauzalnym bez wcześniejszego wywiadu poważnych chorób sercowo-naczyniowych, dokonano ultrasonograficznych pomiarów IMT oraz AP w tętnicy szyjnej wspólnej i obecności CAC za pomocą wielorzędowej tomografii komputerowej. Równolegle oceniono profil lipidowy, parametry zaburzeń gospodarki mineralnej oraz stężenie estradiolu, progesteronu i testosteronu w surowicy.

Wyniki: Występowanie zwapnień w tętnicach wieńcowych stwierdzono u 72% badanych kobiet. Średni wskaźnik uwapnienia wynosił 770 ±1065 jednostek Agatstona, średnia IMT 0,94 ±0,23 mm. Pacjentki, u których

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nie stwierdzano zwapnień naczyniowych miały mniejszą IMT. Stężenie estradiolu w surowicy kobiet bez obecności zwapnień było statystycznie istotnie niższe w porównaniu z pacjentkami chorymi, u których stwierdzano zwapnienia (28,2 ±8,2 *vs* 61,5 ±18,4 pg/mL). Stwierdzono również tendencję do większego stężenia estradiolu u dializowanych chorych ze zmianami miażdżycowymi w tętnicy szyjnej wspólnej. Wykazano silne korelacje pomiędzy stężeniem estradiolu a wskaźnikiem uwapnienia tętnic wieńcowych, IMT oraz obecnością i grubością AP. Nie stwierdzono zależności pomiędzy parametrami antropometrycznymi, innymi ocenianymi parametrami laboratoryjnymi, stężeniem progesteronu, testosteronu a obecnością zmian naczyniowych w badanej populacji.

Wyniki: Wyniki badań nie potwierdzają koncepcji kardioprotekcyjnego działania endogennych estrogenów u przewlekle dializowanych chorych w okresie pomenopauzalnym.

Słowa kluczowe: estrogeny, menopauza, hemodializy, powikłania sercowo-naczyniowe.

Introduction

Cardiovascular calcification and atherosclerosis are strong predictors of cardiovascular events in patients with end-stage renal disease (ESRD) [1]. Some studies have shown that vascular calcification (VC) in chronic kidney disease can be classified on a morphologic and clinical basis as intimal (i.e. calcification of the atherosclerotic plaque) and medial [2], others have proposed that vascular calcification represents a more advanced atherosclerosis process and involves both layers of the vessel wall [3]. Apart from traditional Framingham risk factors for cardiovascular disease like total cholesterol, LDL cholesterol or high blood pressure, there is a number of specific factors, e.g. serum calcium, phosphate and parathyroid hormone tightly related to the risk of vascular calcification (VC) complications in chronic dialysis patients [4, 5].

It is of note that in all studies, including our own observations, a group of patients with ESRD was identified that did not develop VC and showed no significant progression to VC overt time on dialysis [6–8]. It seems therefore important to search for any conditions which prevent development of cardiovascular pathology among patients with ERSD.

Numerous studies have showed that the incidence of cardiovascular disease significantly differs between men and women [9] and the incidence of atherosclerotic disease is low in premenopausal and rises in postmenopausal women. That latter process can be retarded by the introduction of the estrogen replacement therapy [10–13]. Therefore, estrogens seem to play a protective role against the development of cardiovascular complications. The protective effects of estrogens may be due to their beneficial influence on the lipid profile, antioxidant activity, enhanced fibrinolysis and direct actions on the vasculature [14, 15]. On the other hand, the recent evidence from several controlled trials [16, 17] suggests, however, in contrast to some observational studies [18, 19] that the hormonal replacement therapy (HRT) is associated with increased cardiovascular events. The experience with HRT in ESRD women has been very limited to date [20, 21]. Since the effects of endogenous estrogens on cardiovascular damage, including the patients with ESRD have not been fully clarified we decided to investigate the relationship between cardiovascular disease surrogate markers such as coronary artery calcification (CAC), common carotid artery intima media thickness (CCA-IMT), the presence and thickness of atherosclerotic plaques in carotid artery (AP) and serum level of sex hormones in postmenopausal haemodialysis (HD) women.

Material and methods

For this cross-sectional study 22 non-smoking postmenopausal HD women non-menstruating for at least two years prior to the study (mean age 56.1 ±8.3 yrs), without any history of major cardiovascular complications (cardiac infarction, cerebrovascular events, symptoms of intermittent claudication or advanced heart failure) and with well-controlled hypertension were qualified. The demographic data, including age at enrolment, gender, aetiology of ESRD, dialysis vintage were collected. The causes of renal failure were chronic glomerulopathies in 11 cases, diabetic nephropathy in 2, polycystic kidney diseases in 2, tubulointerstitial nephritis in 4, and were unknown in 3 patients. The details of antihypertensive treatment were obtained from the patients' drug charts. All the patients were treated with various combinations of angiotensin-converting enzyme inhibitors, angiotensin II receptor antagonists, calcium antagonists, α- and β-blockers, achieving satisfactory blood pressure control, below 150/80 mm Hg before an HD session. Most women (82%) were also treated with statins. None were treated with sex hormones in the past.

The patients had been on chronic dialysis for at least 6 months (mean time on HD 51.3 ±11.5 months) and were treated with conventional haemodialysis, thrice weekly for 4-5 hours with a low-flux synthetic, polysulfone membrane dialyzers (F-series, Fresenius Medical Care AG, Bad Homburg Germany). Kt/V was > 1.2 in all patients. The standard bicarbonate dialysis bath (Na 138, K 2.5, Ca 1.25 and HCO_3 31 mmol/L) was used. The patient received calcium carbonate as a standard phosphate binder throughout the study period (median dose 3.0 g/day) and were treated with sevelamer hydrochloride only as a rescue binder if the serum phosphorus exceeded

1.8 mmol/L. Ten patients were on oral active vitamin D_3 (alphacalcidol; dose range 0.25-1 µg/day).

Imaging procedures. Multi-slice computed tomography, common carotid artery intima media thickness and thickness of the atherosclerotic plaques

Multi-slice CT scanning of the thorax was performed using General Electric Medical Systems Lightspeed 16 scanner to determine coronary artery calcification. The acquisition parameters were as follows: 120 KVp, 350 mA, slice with 2.5 mm/8i. Data were reconstructed with a standard algorithm using a 512x512 matrix, 50 cm scan field of view and 25 cm display field of view. The system was synchronized with the cardiac cycle to trigger scanning during the diastolic phase. All pixels with an intensity ≥ 130 Hounsfield units (HU) were counted and data were analyzed using CardIQ Smart Score software (GE). CAC score (CACS) was determined using the Agatston scoring system, CACS thresholds < 100 were assessed as no significant calcification [22].

Carotid ultrasonography. Ultrasound examinations were performed with GE "VIVID 7 PRO" machine using 5-14 MHz linear high-resolution probe. Each patient was examined in the supine position in a semi-dark room. The ultrasonography and CCA-IMT measurements were performed on the same mid-week dialysis day. The carotid arteries on both sides were investigated by the same expert radiologist who was unaware of the purpose of the study and the results of laboratory measurements. CCA-IMT was defined as a low-level echo grey band that does not project into the arterial lumen and was measured at the diastolic phase as a distance between the leading edge of the first and second echogenic line. CCA-IMT was measured on the longitudinal views of the far wall of the distal segment of the common carotid artery, the carotid bifurcation and the initial tract of the internal carotid artery on both sides. Measurements were performed 0.5, 1 and 2 cm below and above the bifurcation in a plaque-free arterial segment. The mean values were taken as CCA-IMT and considered abnormal when > 0.82 mm [23]. Carotid plaques were defined (and counted) either as faint grey echoes (soft plaques) or bright white echoes (calcified plaques) protruding into the arterial lumen. Plaque thickness was measured in a suitable longitudinal or transverse view. Plaque score was computed by summing maximum thickness in millimetres of plaques in each segment on both sides [24].

Laboratory assessment

Blood samples for the biochemical and hormonal profile measurements were drawn prior to the mid-week dialysis session. Serum calcium, phosphorus, albumin, cholesterol, triglycerides and HDL cholesterol were measured with routine laboratory methods using an automated analyzer (Olympus AU560, Hamburg, Germany). LDL cholesterol was calculated from the Friedewald formula. Serum estradiol, progesterone, testosterone and PTH were measured by an immunoenzymatic assay from Roche Diagnostic GmbH, Mannheim, Germany. The patients were informed about the aim and design of the study and gave written consent prior to it. The study protocol was accepted by the local Ethics Committee.

Statistical analysis

Mean values and standard deviation were calculated for all assessed groups of studied subjects. Upon confirmation of normal distribution with Shapiro-Wilk test, t-Student test for unpaired data was used to assess significance of differences between the means. Significance of the differences in parameter distribution was measured with chi-square or Fisher exact test. The power of associations between continuous variables was calculated with Pearson's linear regression equations or for non-normally distributed parameters – with Spearman method. The level of statistical significance was set at $p < 0.05$.

Results

The CACS in the group of postmenopausal HD women ranged widely from 0 to 4582 HU. The coronary artery calcifications were detectable (CACS ≥ 100) in 72% of examined women (16/22). Among women with calcification (CACS ≥ 100) six females had very advanced lesions (> 1000 Agatston units). The mean CACS in our HD women was 770 ±1065. The mean thickness of CCA-IMT was 0.94 ±0.23 mm and 10 of the patients had CCA-IMT within the normal range. The plaques in the common carotid arteries were visualized in 18 patients (81%) with a maximum thickness of 2.8 mm. The mean thickness of the AP was 1.68 ±0.63 mm. The same women who did not develop vascular calcification had lower CCA-IMT than those with CAC (0.73 ±0.15 *vs* 1.01 ±0.22 mm; $p < 0.009$) and only two of them had detectable plaques in the common carotid arteries. In women with CAC < 100 the serum level of estradiol was significantly lower than in HD women with significant coronary calcification (28.2 ±8.2 *vs* 61.5 ±18,4 pg/mL, $p < 0.03$). We observed a tendency for higher serum estradiol in HD women who showed the markers of atherosclerosis in the common carotid artery in comparison to women with normal CCA-ITM (59.2 ±17.0 pg/mL *vs* 44.3 ±25.5 pg/ml), $p < 0.06$. The strong correlations between the serum level of estradiol and, respectively CACS ($R = 0.547$, $p < 0.01$; Fig. 1), CCA-IMT ($R = 0.47$, $p < 0.05$; Fig. 2) and presence and thickness of AP ($R = 0.56$, $p < 0.01$, Fig. 3) were

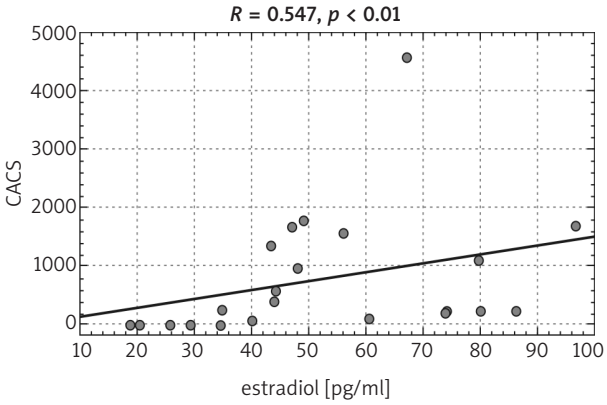

Fig. 1. A correlation between the serum level of estradiol and coronary artery calcium score (CACS) in postmenopausal haemodialysis women

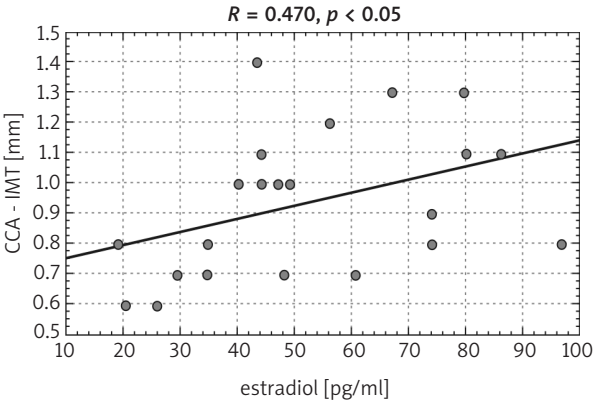

Fig. 2. A correlation between the serum level of estradiol and common carotid artery intima media thickness (CCA-IMT) in postmenopausal haemodialysis women

observed. We did not notice any significant relations between anthropometric parameters, total serum cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, total calcium, phosphorus, Ca x P, serum albumin, serum levels of progesterone and testosterone in dialysis women with or without surrogate markers of CV complications such as high CCA-ITM, presence of AP or CAC (Table I).

Discussion

In the general population, the incidence of cardiovascular disease differs significantly between men and women, largely due to differences in risk factor prevalence and sex hormone status [25]. In patients with renal disease, the cardiovascular risk is extremely high. As we have shown in our previous study, the prevalence of coronary artery calcification and markers of atherosclerosis is higher in dialysed men than dialysed women [26]. In this study we assessed the relations between the serum levels of sex hormones (estradiol, progesterone and testosterone) and the presence of atherosclerosis markers and CAC in postmenopausal HD women. There has been very limited evidence on the effects of the HRT in dialysis women on the cardiovascular risk and such serum biomarkers of cardiovascular disease as serum lipids [20, 21]. Furthermore, there have been no studies assessing such effects on specific markers of cardiovascular complications like CAC and CCA-IMT in women with ESRD.

In our study, those postmenopausal women who did not develop coronary calcification and had no symptoms of atherosclerosis showed lower endogenous estradiol levels than patients with a high CAC score and presented markers of atherosclerosis in the common carotid artery. Interestingly, we did not notice any correlations between lipid profiles and sex hormones profile in our population, but this could be due to the fact that

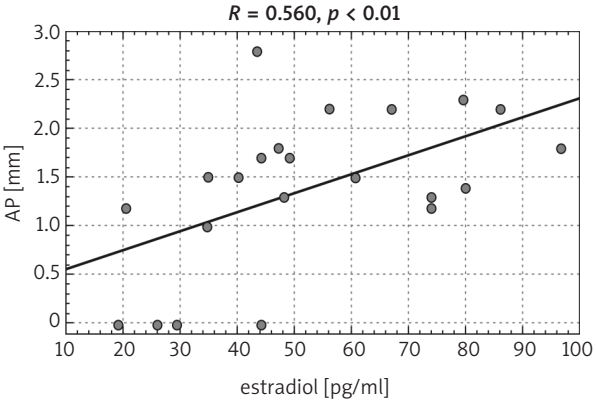

Fig. 3. A correlation between the serum level of estradiol and thickness of atherosclerotic plaques (AP) in postmenopausal haemodialysis women

most of our women were chronically treated with lipid-lowering agents. Our results therefore contrast with the hypothesis coming from the observational studies carried out in the general population [25] that estrogens may have protective effects on the cardiovascular system in postmenopausal women with ESRD.

In experimental animals, estrogen treatment consistently reduced the development of carotid intimal medial lesions induced by a mechanical injury or atherogenic diet [27, 28]. The potential cardioprotective effects of estrogens in renal disease was investigated by Gross et al. who looked at the effects of substitution of estrogens in ovariectomized rats on structural parameters of heart and aorta in a model of renal insufficiency in uninephrectomized animals [29]. That study showed that in ovariectomized rats with moderate impairment of renal function, administration of estrogens prevented cardiac damage but failed to prevent the development of arterial pathologies manifested by increased wall thickness of intramyocardial arteries and of aorta. Furthermore, no positive effect on lipid levels was observed in that

Tab. I. Anthropometric parameters, vascular damage markers, serum estradiol, progesterone, testosterone, haemoglobin, albumin, lipids and calcium-phosphate balance parameters in postmenopausal HD women free of coronary artery calcification (CAC) and with detectable CAC

Parameter	Unit	Mean ± SD		*p-value*
		free of CAC (*n* = 6)	with CAC (*n* = 16)	
age	years	56.8 ±7.8	55.7 ±8.9	ns
dialysis vintage	months	54.7 ±8.2	49.6 ±112.8	ns
BMI	kg/m^2	28.0 ±6.4	24.1 ±4.3	ns
CACS	Agatston units	n/a	1054 ±1128	**n/a**
CCA-IMT	mm	0.73 ±0.15	1.01 ±0.22	**0.009**
AP	mm	0.62 ±0.69	1.68 ±0.63	**0.003**
estradiol	pg/mL	28.2 ±8.2	61.5 ±18.4	**0.0004**
progesterone	pg/mL	0.48 ±0.2	0.44 ±0.2	ns
testosterone	pg/mL	0.26 ±0.2	0.28 ±0.2	ns
PTH	pg/mL	188.3 ±91.9	298.2 ±206.9	ns
haemoglobin	g/dL	11.3 ±0.8	10.9 ±1.2	ns
total cholesterol	mmol/L	4.7 ±0.4	5.0 ±1.2	ns
triglycerides	mmol/L	2.25 ±0.7	2.2 ±0.9	ns
LDL-cholesterol	mmol/L	2.6 ±0.7	3.1 ±1.1	ns
HDL-cholesterol	mmol/L	2.65 ±0.75	3.12 ±0.7	ns
total calcium	mg/dL	2.26 ±0.75	2.3 ±0.2	ns
phosphorus	mg/dL	1.63 ±0.3	1.66 ±0.6	ns
Ca x P	mg^2/dL^2	3.67 ±0.33	3.77 ±1.43	ns
albumin	g/L	32.7 ±14.6	33.5 ±9.7	ns

CACS – coronary artery calcification score; CCA-IMT – common carotid artery intima-media thickness; AP – thickness of atherosclerotic plaques in common carotid artery

study. Tatchum-Talom et al. in their experimental study observed that the estrogen treatment increased aortic stiffness [30]. It is well known that the presence of CAC in dialysis patients tightly correlates with arterial stiffness [31]. Our observation that a higher level of endogenous estrogens in postmenopausal HD women is correlated with the development of the cardiovascular calcification and atherosclerosis confirms the findings from experimental studies [29].

In a human study, the effect of exogenous estrogens in preventing cardiovascular disease is still a matter of controversy. There is some evidence that replacing estrogens may reduce the progression of subclinical atherosclerosis assessed by the changes of thickness of intima-media complex in the common carotid artery in healthy postmenopausal women without any pre-existing cardiovascular disease and with a good kidney function over a 2-year period [32]. The beneficial effect of estrogens on the CAC score in postmenopausal women with a good kidney function was also reported by Akhrass et al. [33] and Manson et al. [34].

In contrast, Herrington et al. revealed the lack of effects of the estrogen replacement therapy on the progression of coronary artery atherosclerosis in postmenopausal women [35]. The incidence of the ischemic stroke as a consequence of carotid arterial atherosclerosis was not reduced in women participating in the large Women's Health Initiative trial that assessed the influence of postmenopausal hormone therapy on the cardiovascular risk [17, 36]. In this pivotal study, women in whom the hormone replacement therapy (HRT) was initiated closer to menopause tended to show a reduced coronary heart disease risk compared with women who started the therapy farther from menopause. In our study, we did not observe any relation between other sex hormones such as progesterone or testosterone and atherosclerotic or calcific lesions in postmenopausal HD women. The lack of such relationships between those hormones and cardiovascular complications in the general population of postmenopausal women was also reported by some other authors [37–39], however others such as Bernini et al. found that androgens in the physiological range may be correlated with a lower risk of carotid artery atherosclerosis [40]. In summary, our results do not support the cardiovascular protective role of endogenous estrogens in postmenopausal women on the chronic dialysis therapy.

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