Sex hormone levels and the presence of atherosclerosis and coronary calcification in postmenopausal women with chronic kidney disease stage 3-5

Stężenie hormonów płciowych a obecność zmian miażdżycowych oraz kalcyfikacji naczyniowej u kobiet w okresie menopauzy w 3.–5. stadium przewlekłej choroby nerek

Ilona Kurnatowska[1], Piotr Grzelak[2], Magdalena Kaczmarska[2], Anna Masajtis-Zagajewska[1], Ludomir Stefańczyk[2], Michał Nowicki[1]

[1]Department of Nephrology, Hypertension and Kidney Transplantation;
head of Department: prof. dr hab. n. med. Michał Nowicki
[2]Department of Radiology and Diagnostic Imaging, Medical University of Łódź, Poland;
head of Department: prof. dr hab. n. med. Ludomir Stefańczyk

Przegląd Menopauzalny 2012; 2: 81–86

Summary

Background: It has recently been found that both estrogens and testosterone may inhibit vascular calcification but the role of sex hormones in the development of cardiovascular complications in chronic kidney disease (CKD) is still unclear.

Aim of the study: The aim of the study was to assess the relations between sex hormones, coronary artery calcification (CAC) and atherosclerosis in postmenopausal women with CKD stage 3-5.

Material and methods: In a cross-sectional study, serum estradiol, testosterone, dehydroepiandrosterone sulfate (DHEA), 25-hydroxyvitamin D3 (25-OH-D3), mineral parameters and lipids were measured in 36 Caucasian, non-dialysed amenorrheic women (mean age 56.8 ±11.4 yrs), with CKD stage 3-5 (mean eGFR 20.8 ±8.8 ml/min/m^2), without any history of major cardiovascular events. CAC was measured with multidetector computed tomography and common carotid artery intima media thickness (CCA-IMT) with ultrasound.

Results: The prevalence of CAC (CAC score > 30 Agatston units) was 44.4%. Mean CAC score in women with detectable calcification was 177.3 ±268.4. The women who did not develop vascular calcification had also lower CCA-IMT (0.65 ±0.13 *vs.* 0.88 ±0.12 mm, $p < 0.0004$). In women with CAC, serum estradiol was significantly higher than in those without CAC (30.1 ±14.2 vs. 14.7 ±7.44 pg/mL, $p < 0.001$). Similarly, a testosterone level in women with CAC was higher than in those without CAC (5.4 ±4.2 vs. 2.3 ±1.3 pg/mL, $p < 0.006$) and the same was observed for DHEA-s (100.5 ±67.6 *vs.* 60.3 ±56.4 µg/dL, respectively; $p < 0.05$). Serum 25-OH-D3 was similar in both groups. There was a significant correlation between the serum level of estradiol and CAC score ($R = 0.533$, $p < 0.05$) and between the serum estradiol level and total cholesterol ($R = 0.505$, $p < 0.05$), LDL-Ch ($R = 0.585$, $p < 0.05$), phosphorus level ($R = 0.4$, $p < 0.03$) and calcium-phosphorus index (Ca x P: $R = 0.44$, $p < 0.04$) and between serum testosterone and total cholesterol ($R = 0.5$, $p < 0.05$).

Conclusion: Our results do not support the inhibitory effects of endogenous sex hormones on vascular calcification in postmenopausal women with advanced chronic kidney disease.

Key words: vascular calcification, renal insufficiency, sex hormones.

Streszczenie

Wstęp: Rola endogennych hormonów płciowych w rozwoju powikłań naczyniowych, takich jak miażdżyca czy zwapnienie naczyń u kobiet z przewlekłą chorobą nerek (PChN), nie została dotąd jednoznacznie ustalona.

Cel pracy: Celem niniejszej pracy była ocena zależności pomiędzy stężeniem hormonów płciowych a występowaniem wskaźników miażdżycy i zwapnienia naczyń u kobiet w 3.–5. stadium PChN w okresie pomenopauzalnym.

Address for corespondence:
Michał Nowicki, Department of Nephrology, Hypertension and Kidney Transplantation, Medical University of Łódź, ul. Kopcińskiego 22, 91-156 Łódź, Poland, tel. +48 42 677 67 09, fax: +48 42 678 36 32, Email: nefro@wp.pl

Materiał i metody: W przekrojowym badaniu, do którego zakwalifikowano 36 kobiet z przewlekłą chorobą nerek [filtracja kłębuszkowa (eGFR) <60 ml/min/m^2 pow. ciała] w okresie pomenopauzalnym, bez stwierdzenia we wcześniejszym wywiadzie poważnych chorób sercowo-naczyniowych, dokonano ultrasonograficznych pomiarów kompleksu *intima media* (IMT) w tętnicy szyjnej wspólnej i obecności zwapnień w naczyniach wieńcowych (CAC) za pomocą wielorzędowej tomografii komputerowej. Równolegle oceniono profil lipidowy, parametry zaburzeń gospodarki mineralnej, stężenie 25-hydroksywitaminy D_3 (25-OH-D_3) oraz stężenie estradiolu, testosteronu i siarczanu dehydroepiandrosteronu (DHEA-S) w surowicy.

Wyniki: Występowanie zwapnień w tętnicach wieńcowych (wskaźnik CAC > 30 jednostek Agatstona) stwierdzono u 44,4% badanych kobiet. Średni wskaźnik uwapnienia wynosił 177,3 ±268,4 jednostek Agatstona. Pacjentki, u których nie stwierdzano zwapnień naczyniowych, miały mniejszą IMT (0,65 ±0,13 *vs* 0,88 ±0,12 mm, $p < 0,0004$). Stężenie estradiolu w surowicy kobiet bez obecności zwapnień było statystycznie istotnie niższe w porównaniu z chorymi, u których stwierdzano zwapnienia (14,7 ±7,4 *vs* 30,1 ±14,2 pg/ml, $p < 0,001$). Podobną zależność wykazano dla stężenia testosteronu (2,3 ±1,3 *vs* 5,4 ±4,2 pg/ml, $p < 0,006$) oraz DHEA-S (60,3 ±56,4 *vs* 100,5 ±67,6 µg/dl, $p < 0,05$). Stężenie 25-OH-D_3 było porównywalne w obu grupach. Wykazano korelację pomiędzy stężeniem estradiolu a CACS ($R = 0,533$, $p < 0,05$) oraz pomiędzy stężeniem estradiolu i całkowitego cholesterolu ($R = 0,505$, $p < 0,05$), LDL-Ch ($R = 0,585$, $p < 0,05$), fosforu ($R = 0,4$, $p < 0,03$), a także iloczynem wapniowo-fosforowym (Ca x P: $R = 0,44$, $p < 0,04$) oraz pomiędzy stężeniem testosteronu i całkowitym cholesterolem ($R = 0,5$, $p < 0,05$).

Wnioski: Wyniki badań nie potwierdzają kardioprotekcyjnego działania endogennych hormonów płciowych u kobiet z upośledzoną czynnością nerek w okresie pomenopauzalnym.

Słowa kluczowe: kalcyfikacja naczyniowa, hormony płciowe, niewydolność nerek.

Introduction

Chronic kidney disease (CKD) has been associated with a very high risk of cardiovascular complications [1, 2]. Both vascular calcification and atherosclerosis are strong predictors of cardiovascular events in CKD patients [3]. CKD patients have a heavy burden of traditional cardiovascular risk factors in addition to a range of nontraditional risk factors such as inflammation and abnormal metabolism of calcium and phosphate [4, 5]. It is of note that in all studies, including our own observations, a group of patients with CKD was identified who did not develop VC and showed no significant progression to VC with the duration of illness [6-8]. It seems therefore important to search for any conditions and their biomarkers which protect from development of cardiovascular pathology among patients with CKD. Numerous studies 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. The 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] suggested that in contrast to some observational studies [18, 19] the hormonal replacement therapy (HRT) was associated with increased cardiovascular events. The experience with HRT in women with more advanced CKD has been very limited to date [20, 21].

Apart from estrogens, the androgen status may play an important role in determining the cardiovascular risk in postmenopausal women. The association of endogenous androgens with cardiovascular risk factors and the incidence of cardiovascular events are not clear. Some studies indicated protective effects of endogenous androgens on the vessels' wall [22] but some showed opposite effects [23]. Our previous research showed no protective effects of endogenous sex hormone on the development of vessel calcification among postmenopausal haemodialysis women [24]. The effects of endogenous sex hormones on cardiovascular damage in postmenopausal non-dialysis women with CKD stage 3-5 have not been fully clarified. The aim of this study was to investigate the relationship between surrogate markers of cardiovascular disease such as coronary artery calcification (CAC), common carotid artery intima media thickness (CCA-IMT) and serum level of sex hormones in postmenopausal women with eGFR < 60 ml/min/m^2.

Material and methods

For this cross-sectional study, 36 non-smoking, non-dialysis women with CKD stage 3-5, non-menstruating for at least two years prior to the study with mean age of 56.8 ±11.4 yrs, without 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 causes of renal failure were chronic glomerulopathies in 10 cases, diabetic nephropathy in 7, polycystic kidney diseases in 4, tubulointerstitial nephritis in 6, hypertensive

nephropathy in 5 and unknown in 4 patients. 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 140/90 mm Hg). All patients were treated with statins. None were treated with sex hormones in the past and with vitamin D_3 or steroids in the last two years before examination. Five patients received calcium carbonate as a standard phosphate binder.

Imaging procedures. Multislice computed tomography, common carotid artery intima media thickness

Multislice 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 < 30 were assessed as no significant calcification [25].

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 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 [26].

Laboratory assessment

Blood samples for the biochemical and hormonal profile measurements were drawn after fasting, in the morning. 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 and dehydroepiandrosterone sulfate (DHEA-S) were measured by a radioimmunoassay from Immunotech A Beckman Coulter Company, Marseille Cedex, France, free testosterone by enzyme immunoassay from NovaTec Immunodiagnostica GmbH, Dietzenbach, Germany and 25-OH-D3H by enzyme immunoassay 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 the differences between the means. Significance of the differences in parameters' 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. A level of statistical significance was set at < 0.05.

Results

The CACS in the group of postmenopausal non-dialyzed CKD women ranged widely from 0 to 1067 HU. The coronary artery calcifications were detectable (CACS ≥ 30) in 44.4% of examined women (16/36). The mean CACS in CKD women was 177.3 ±268.4. The mean thickness of CCA-IMT was 0.78 ±0.17 mm and 11 of the patients had CCA-IMT within the normal range (only women with CAC). The same women who did not develop vascular calcification had lower CCA-IMT than those with CAC (0.65 ±0.13 vs. 0.88 ±0.12 mm; $p < 0.0004$). In women with CAC < 30 the serum level of estradiol was significantly lower than in CKD women with significant coronary calcification (14.7 ±7.4 vs. 30.1 ±14.2 pg/mL, $p < 0.001$). The same relationships were noticed for the testosterone level (2.3 ±1.3 vs. 5.4 ±4.2 pg/mL, $p < 0.006$) and DHEA-S (60.3 ±56.4 vs. 100.5 ±67.6 µg/dL, $p < 0.05$). The correlation between the serum level of estradiol and CACS ($R = 0.533$, $p < 0.05$; Fig. 1) was observed. A higher hsCRP level was observed in CKD women with CAC (6.2 ±5.2 vs. 2.0 ±2.2 mg/L, $p < 0.01$). The levels of vitamin D, calcium and phosphorus were similar in

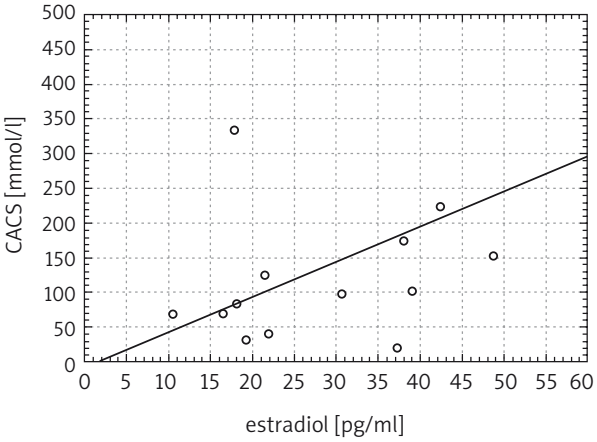

Fig. 1. A correlation between serum level of estradiol and coronary artery calcium score (CACS) in postmenopausal women with chronic kidney disease

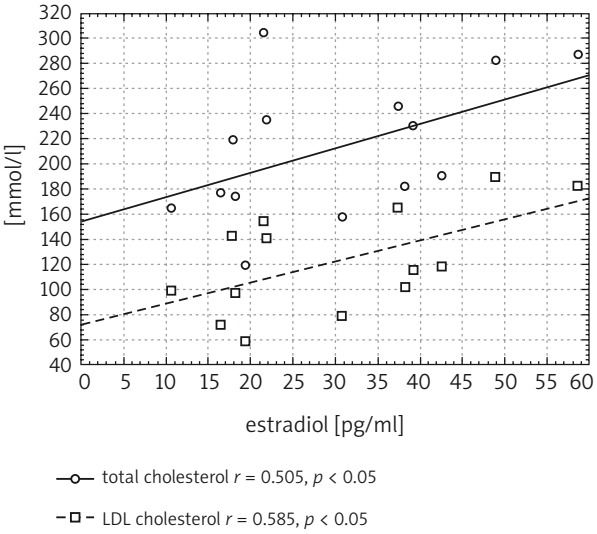

Fig. 2. A correlation between serum level of estradiol and serum total cholesterol and LDL-cholesterol in postmenopausal women with chronic kidney disease with coronary calcification

both examined groups (Table I), but in patients with CAC a higher PTH level was observed (261.9 ±135.4 vs. 151.0 ±153.0 pg/mL, $p < 0.05$). In CAC patients we also find a positive correlation between estradiol level and total cholesterol ($R = 0.505$, $p < 0.05$, Fig. 2), LDL-cholesterol ($R = 0.585$, $p < 0.05$, Fig. 2), serum phosphorus level ($R = 0.4$, $p < 0.03$) and calcium-phosphorus index (Ca x P: $R = 0.44$, $p < 0.04$) and between serum testosterone and total cholesterol level ($R = 0.5$, $p < 0.05$). We did not observe such correlation in women free of calcification.

Tab. I. Anthropometric measures, vascular damage markers, serum estradiol, testosterone, and dehydroepiandrosterone sulfate (DHEA), 25-hydroxyvitamin D3 (25-OH-D3), hsCRP, PTH, lipids and calcium-phosphate parameters in postmenopausal HD women free of coronary artery calcification (CAC) and with detectable CAC

Parameter	Unit	Mean ± SD		*p value*
		free of CAC ($n = 20$)	with CAC ($n = 16$)	
Age	years	58.7 ±7.9	61.8 ±8.0	ns
BMI	kg/m²	27.6 ±6.8	30.0 ±5.4	ns
eGFR	ml/min/m²	25.6 ±11.4	16.1 ±6.4	**0.01**
CACS	Agatston units	n/a (<30)	177.3 ±268.4	n/a
CCA-IMT	mm	0.65 ±0.13	0.88 ±0.12	**0.0004**
Estradiol	pg/mL	14.7 ±7.44	30.1 ±14.2	**0.001**
DHEA-S	µg/dL	60.3 ±56.4	100.5 ±67.6	**0.05**
Testosterone	pg/mL	2.3 ±1.3	5.4 ±4.2	**0.006**
25-OH-D3	ng/mL	23.2 ±13.3	21.9 ±9.8	ns
PTH	pg/mL	151.0 ±153.0	261.9 ±135.4	**0.05**
hsCRP	mg/L	2.0 ±2.2	6.2 ±5.2	**0.01**
Total cholesterol	mmol/L	188.9 ±16.7	212.1 ±54.8	ns
Triglycerides	mmol/L	156.1 ± 80.2	160.7 ±73.7	ns
LDL-cholesterol	mmol/L	107.3 ±14.8	122.6 ±40.9	ns
HDL-cholesterol	mmol/L	57.9 ±13.5	59.7 ±22.1	ns
Total calcium	mg/dL	2.4 ±0.1	2.4 ±0.3	ns
Phosphorus	mg/dL	1.4 ±0.4	1.3 ±0.2	ns
Ca x P	mg²/dL²	3.78 ±1.39	3.77 ±1.43	ns

CACS – coronary artery calcification score; CCA-IMT – common carotid artery intima-media thickness; eGFR – estimated glomerular filtration rate; 25-OH-D3 – 25-hydroksyvitamin D3; hsCRP – high-sensitivity C-reactive protein.

Table I presents the anthropometric measures, lipid profile, calcium-phosphorus parameters, and serum levels of PTH, hsCRP, vitamin D, estradiol, testosterone and DHEA-S in women with CAC and without this complication.

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 [27]. There is no information about the level of endogenous sex hormones and markers of atherosclerosis or vessel calcifications in postmenopausal non-dialyzed women with CKD. The present study demonstrates significant associations of circulating estradiol and androgens (DHEA-S and testosterone) and markers of vascular wall damage in postmenopausal, non-dialysis women with GFR < 60 ml/min/kg^2. The study confirms our previous observation of no protective effect of endogenous estrogens on the development of vascular calcification and atherosclerosis among postmenopausal dialysis women [24]. In our study, those women who did not develop coronary calcification and had no symptoms of atherosclerosis showed a lower serum concentration of endogenous estradiol, testosterone and DHEA-S levels than patients with high CAC score and presented the markers of atherosclerosis in the common carotid artery. Interestingly we noticed positive correlations between serum estrogen level and total cholesterol and LDL-cholesterol and testosterone level and cholesterol. Although these correlations cannot prove any causal relationship they may suggest the associations of higher cardiovascular risk in patients with calcification. Our results therefore may contrast with the hypothesis, coming from the observational studies in the general population of postmenopausal women [27] that estrogens may exert protective effects on the cardiovascular system in postmenopausal CKD women and have a beneficial influence on the lipid profile [14, 15]. The observations that higher endogenous estradiol and increased androgenicity are related to pro-atherogenic lipid profile were reported by Lambrinoudaki et al. [28].

In experimental studies, estrogen treatment consistently reduced the development of carotid intimal medial lesions induced by a mechanical injury or atherogenic diet [29, 30]. The potential cardioprotective effects of estrogens in renal disease was investigated by Gross et al. who looked at the effects of the substitution of estrogens in ovariectomized rats on structural parameters of heart and aorta in a model of renal insufficiency in uninephrectomized animals [31]. 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 the aorta. Furthermore, as in our study, no positive effect on lipid levels was observed in that study. In their experimental study, Tatchum-Talom et al. observed that estrogens treatment increased aortic stiffness [32]. It is well known that the presence of CAC in dialysis patients closely correlates with arterial stiffness [33]. Our observation that a higher level of endogenous estrogens in postmenopausal CKD women was correlated with the development of the cardiovascular calcification and atherosclerosis may confirm the findings from experimental studies [31].

Our study showed that higher androgenicity in postmenopausal CKD women is associated with the presence of CAC and markers of atherosclerosis. The data on the role of androgens in the development of CV complications in the general population have not conferred a clear message. High levels of circulating testosterone in postmenopausal women have been associated with accelerated coronary atherosclerosis [34], as well as with increased prevalence and incidence of coronary heart disease [35]. On the contrary, other studies support the hypothesis that women with a lower level of circulating testosterone have more carotid atherosclerosis compared with women with higher but normal levels of testosterone [23, 36, 37]. There is evidence that DHEA-S produced mainly by the adrenal glands and up to 20% by the ovaries, may have a vasculoprotective role even after the menopause [23, 38]. In line with our results, a recent study in an elderly Japanese population with cardiovascular risk factors revealed protective effects of DHEA-S on IMT only in men, but not in women [39].

In summary, our results do not support the cardiovascular protective role of endogenous estrogens and androgens in postmenopausal women with moderate to advanced chronic kidney disease.

References

1. Vanholder R, Massy Z, Argiles A, et al. Chronic kidney disease as cause of cardiovascular morbidity and mortality. Nephrol Dial Transplant 2005; 20: 1048-56.
2. McCullough PA, Li S, Jurkovitz CT, et al. Kidney Early Evaluation Program Investigators. CKD and cardiovascular disease in screened high-risk volunteer and general populations: the Kidney Early Evaluation Program (KEEP) and National Health and Nutrition Examination Survey (NHANES) 1999-2004. Am J Kidney Dis 2008; 51(4 Suppl 2): 38-45.
3. Goodman WG. Vascular calcification in end-stage renal disease. J Nephrol 2002; 15 (Suppl 6): 82-5.
4. Cozzolino M, Dusso AS, Slatopolsky E. Role of calcium-phosphate product and bone-associated proteins on vascular calcification in renal failure. Clin J Am Soc Nephrol 2001;12: 2511-6.
5. Floege J, Ketteler M. Vascular calcification in patients with end-stage renal disease. Nephrol Dial Transplant 2004; 19 (Suppl): 59-66.
6. Moe SM, O'Neill KD, Resterova M, et al. Natural history of vascular calcifications in dialysis and transplant patients. Nephrol Dial Transplant 2004; 19: 2387-93.

7. Block GA, Spiegel DM, Ehrlich J, et al. Effects of sevelamer and calcium on coronary artery calcification in patients new to hemodialysis. Kidney Int 2005; 68 :1815-24.
8. Kurnatowska I, Grzelak P, Kaczmarska M, et al. Serum osteoprotegerin is a predictor of progression of atherosclerosis and coronary calcification in hemodialysis patients. Nephron Clin Pract 2011; 117: 297-304.
9. Barrett-Connor E. Sex differences in coronary heart disease: why are women so superior? The 1995 Ancel Keys Lecture. Circulation 1997; 95: 252-64
10. Stampfer MJ, Colditz GA, Willett WC, et al Postmenopausal estrogen therapy and cardiovascular disease. Ten-year follow-up from the nurses' health study. N Engl J Med 1991; 325: 756-62.
11. Grady D, Rubin SM, Petitti DB, et al. Hormone therapy to prevent disease and prolong life in postmenopausal women. Ann Intern Med 1992; 117: 1016-37.
12. Dębski R, Paszkowski T, Pawelczyk L, Pertyński T. Terapia hormonalna okresu menopauzalnego – stan wiedzy w 2010 r. Stanowisko Zespołu Ekspertów Polskiego Towarzystwa Menopauzy i Andropauzy. Przegl Menopauz 2010; 3: 121-7.
13. Stachowiak G, Pertyński T. Bezpieczeństwo kardiologiczne terapii hormonalnych okresu menopauzy. Przegl Menopauz 2009; 6: 315-9.
14. Tostes RC, Nigro D, Fortes ZB, Carvalho MH. Effects of estrogen on the vascular system. Braz J Med Biol Res 2003; 36: 1143-58.
15. Sobstyl M, Tkaczuk-Włach J, Robak-Chołubek D, Jakiel G. Miażdżyca a status hormonalny kobiety przed menopauzą. Przegl Menopauz 2009; 4: 244-7.
16. Herrington DM. The HERS trial results: paradigms lost? Heart and Estrogen/progestin Replacement Study. Ann Intern Med 1999; 131: 463-6.
17. Rossouw JE, Anderson GL, Prentice RL, et al. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. JAMA 2002; 288: 321-33.
18. Barrett-Connor E, Bush TL. Estrogen and coronary heart disease in women. JAMA 1991; 265: 1861-7.
19. Kannel WB, Hjortland MC, McNamara PM, Gordon T. Menopause and risk of cardiovascular disease: the Framingham study. Ann Intern Med 1976; 85: 447-2.
20. Matuszkiewicz-Rowińska J, Skórzewska K, Radowicki S, et al. [Hormonal replacement therapy and lipid metabolism in women on hemodialysis with secondary to uremia estrogen deficiency.] Pol Arch Med Wewn 1999; 102: 671-6.
21. Park JS, Jung HH, Yang WS, et al. Effects of hormonal replacement therapy on lipid and haemostatic factors in post-menopausal ESRD patients. Nephrol Dial Transplant 2000; 15: 1835-40.
22. Bernini GP, Moretti A, Sgró M, et al. Influence of endogenous androgens on carotid wall in postmenopausal women. Menopause 2001; 8: 43-50.
23. Creatsa M, Armeni E, Stamatelopoulos K, et al. Circulating androgen levels are associated with subclinical atherosclerosis and arterial stiffness in healthy recently menopausal women. Metabolism 2012; 61: 193-201.
24. Kurnatowska I, Grzelak P, Kaczmarska M, et al. Relations between serum sex hormone levels and biomarkers of atherosclerosis and mineral disturbances in postmenopausal haemodialysis women. Przegl Menopauz 2010; 5: 324-9.
25. Muntner P, Ferramosca E, Bellasi A, et al. Development of a cardiovascular calcification index using simple imaging tools in haemodialysis patients. Nephrol Dial Transplant 2007; 22: 508-14.
26. Aminbakhsh A, Mancini GB. Carotid intima-media thickness measurements: what defines an abnormality? A systematic review. Clin Invest Med 1999; 22: 149-7.
27. Mendelsohn ME, Karas RH. The protective effects of estrogen on the cardiovascular system. N Engl J Med 1999; 340: 1801-11.
28. Lambrinoudaki I, Christodoulakos G, Rizos D, et al. Endogenous sex hormones and risk factors for atherosclerosis in healthy Greek postmenopausal women. Eur J Endocrinol 2006; 154: 907-16.
29. Oparil S, Chen SJ, Chen YF, et al. Estrogen attenuates the adventitial contribution to neointima formation in injured rat carotid arteries. Cardiovasc Res 1999; 44: 608-14.
30. Miller VM, Duckles SP. Vascular actions of estrogens: functional implications. Pharmacol Rev 2008; 60: 210-41.
31. Gross ML, Ritz E, Korsch M, et al. Effects of estrogens on cardiovascular structure in uninephrectomized SHRsp rats. Kidney Int 2005; 67: 849-57.
32. Tatchum-Talom R, Martel C, Marette A. Influence of estrogen on aortic stiffness and endothelial function in female rats. Am J Physiol Heart Circ Physiol 2002; 282: 491-8.
33. Covic A, Kanbay M, Voroneanu L, et al. Vascular calcification in chronic kidney disease. Clin Sci 2010; 28: 111-21.
34. He H, Yang F, Liu X, et al. Sex hormone ratio changes in men and postmenopausal women with coronary artery disease. Menopause 2007; 14: 385-90.
35. Braunstein GD, Johnson BD, Stańczyk FZ, et al. Relations between endogenous androgens and estrogens in postmenopausal women with suspected ischemic heart disease. J Clin Endocrinol Metab 2008; 93: 4268-75.
36. Sievers C, Klotsche J, Pieper L, et al. Low testosterone levels predict all-cause mortality and cardiovascular events in women: a prospective cohort study in German primary care patients. Eur J Endocrinol 2010; 163: 699-708.
37. Vitale C, Fini M, Speziale G, Chierchia S. Gender differences in the cardiovascular effects of sex hormones. Fundam Clin Pharmacol 2010; 24: 675-85.
38. Akishita M, Hashimoto M, Ohike Y, et al. Association of plasma dehydroepiandrosterone-sulfate levels with endothelial function in postmenopausal women with coronary risk factors. Hypertens Res 2008; 31: 69-74.
39. Yoshida S, Aihara K, Azuma H, et al. Dehydroepiandrosterone sulfate is inversely associated with sex-dependent diverse carotid atherosclerosis regardless of endothelial function. Atherosclerosis 2010; 212: 310-5.