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Low density lipoprotein cholesterol decreases vascular cell adhesion molecule-1 in postmenopausal women

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ABSTRACT

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In premenopausal women cardiovascular disease is rarely encountered, but after menopause the prevalence of cardiovascular disease increases drastically. There are several risk factors for cardiovascular disease, known as traditional risk factors, among others body fat concentration, age, duration of menopause, body mass index (BMI), and estradiol concentration. Cardiovascular disease is considered as an inflammatory disorder, in which adhesion molecules play an important role. Vascular cell adhesion molecule-1 (VCAM-1) is one of the adhesion molecules with an important role in the atherosclerotic process. The aim of this study was to determine the relationship of risk factors affecting the expression of VCAM-1 in postmenopausal women. This study was a cross-sectional study involving 182 postmenopausal women in the age range of 47- 60 years, who were residents of Mampang Prapatan subdistrict, South Jakarta. Venous blood samples were obtained for laboratory investigations, viz. fasting blood glucose, total cholesterol, high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, triglycerides, creatinine, serum glutamic oxaloacetic transamirase (SGOT), serum glutamic pyruvic transmirasi (SGPT), bilirubin, total protein, albumin, estradiol and vascular cell adhesion molecule-1 (VCAM-1).A multiple regression analysis was performed on traditional risk factors and their relationship with VCAM-1 concentration. The results showed there were five traditional risk factors influencing VCAM-1 concentration, viz. duration of menopause, BMI, estradiol concentration, HDL cholesterol, and LDL cholesterol. Among these five factors, LDL cholesterol had the greatest influence on VCAM-1 expression (beta coefficient = -0.253 and p=0.001). In conclusion, LDL cholesterol concentration decreased VCAM-1 expression in postmenopausal women.

Key words: VCAM-1, LDL cholesterol, postmenopausal women

INTRODUCTION

Cardiovascular disease (CVD) is rarely found in premenopausal women, but cardiovascular and cerebrovascular disease are the main causes of mortality in postmenopausal women, particularly in developed countries. The prevalence of these two diseases is up to

75%, which is larger than the prevalence of 6-8% for breast cancer.[1,2] In postmenopausal women decreased estrogen levels may result in changes in the distribution of body fat from the gynoid into the android type, decreased glucose tolerance, dyslipidemias, high blood pressure, increased sympathetic tone, endothelial dysfunction, and vascular inflammation.[3-5]

The abnormalities caused by decreased estrogens in postmenopausal women comprise vasomotor changes, i.e. hot flushes on the chest, neck and face, which may be accompanied by anxiety and palpitations. Other manifestations include reproductive tract symptoms, psychological symptoms, cognitive disorders, and increases in chronic degenerative disorders, such as osteoporosis, cardiovascular disease, stroke, and Alzheimer's disease.[6-8]

CVD is considered to be an inflammatory disorder.[9-11] The results of several studies indicate that atherosclerosis is associated with inflammatory processes, formation of reactive oxygen species (ROS) and endothelial dysfunction. Several conditions that are regarded as risk factors for inflammation in the development of cardiovascular disease, include dyslipidemias, comprising increased total cholesterol, low density lipoprotein (LDL) cholesterol, triglycerides, and reduced high density lipoprotein (HDL) cholesterol.[9,10,12] CVD in postmenopausal women is associated with endothelial dysfunction, in which there is a defect in the vascular mechanism that protects the vascular endothelium against physical and chemical stimuli, such as inflammation, procoagulation and vasoconstriction.[3,10] One of the markers of endothelial dysfunction is the vascular cell adhesion molecule-1 (VCAM-1), which plays a role in the development of endothelial dysfunction, along with other adhesion molecules. VCAM-1 plays its role at the time of entry of inflammatory cells into the subendothelial tissues.[13-16] Leukocytes normally circulate within the blood stream, but when there is tissue damage or inflammation, these leukocytes adhere to the endothelium of blood vessels and migrate across the vessel wall into the affected tissues. Leukocyte adherence to blood vessel walls comprises a series of events, namely selectin-mediated rolling, activation mediated by chemoattractants, and adhesion to the vascular endothelium mediated by integrins, which are an immunoglobulin superfamily of counter receptors such as VCAM-1 and intercellular cell adhesion molecule-1 (ICAM-1). Subsequently the leukocytes move between the endothelial cells and migrate into the subendothelial tisues. The aim of the present study was to determine the factors influencing VCAM-1 expression in postmenopausal women.[13-16]

METHODS

Design of the study

This study was of cross-sectional design and was conducted in the area of Mampang Prapatan subdistrict health center, from January until March 2010.

Study subjects

The subjects of this study were postmenopausal women between 47 and 60 years of age, with as inclusion criteria healthy postmenopausal women, with a natural cessation of menstruation of minimally 1 year and less than 10 years, not caused by total hysterectomy, bilateral oophorectomy, irradiation, and chemotherapy, with body mass index (BMI) $\leq$ 35 kg/m^2, not taking medications within the last 6 months (steroids, oral antidiabetics, antihypertensives, antihyperlipidemic drugs, hormonal therapy); laboratory tests: bilirubin $\leq$2 mg/dL, serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transminase (SGPT) within reference range (10–36 U/L and SGPT 7–35 U/L, respectively), no abnormalities on physical examination, such as hepatomegaly, hypertension, disorders of cardiac rhythm,

creatinine ≤1.5 mg/dL, capable of actively walking without aids, capable of communication (able to answer questions by themselves or with assistance from others) and agreeing to participate in the study by signing informed consent after receiving information on the aim of the study. The exclusion criteria were: women with chronic or terminal diseases, such as mammary carcinoma, carcinoma of the cervix, endometrial carcinoma, renal failure, cardiac failure, diabetes mellitus, stroke and myocardial infarction / heart attack, severe psychosis (such as schizophrenia), and mastectomy. The study subjects were selected by multistage cluster random sampling from four *kelurahan* (villages), namely Kuningan Barat, Mampang Prapatan, Tegal Parang dan Pela Mampang, ressorting under Mampang Prapatan subdistrict, South Jakarta.

Sample size

The sample size was calculated using the formula : [17]

$$n = \left[\frac{(Z\alpha + Z\beta)}{0{,}5\ell n(1+r)(1-r)} \right]^2 + 3$$

where:
r = coefficient of correlation between HDL cholesterol and VCAM-1 = 0.798.[15]
Z_α = 1.96 Z_β= 0.842 n= 68
The minimum sample size required was 68 subjects.

Anthropometric and blood pressure measurements

Height and weight were determined on lightly clothed subjects without foot wear. Height was measured by means of a portable Microtoise with a precision of 0.1 cm. Body weight was determined using Sage portable scales with a precision of 0.1 kg. Circumference measurements were by means of a measuring tape to the nearest 0.1 cm. The abdominal circumference taken was the minimal circumference of the abdomen between the midpoint of the lower rib and the superior anterior iliac spine. Hip circumference was the largest circumference between the gluteal muscle dorsally and the pubic symphysis ventrally. BMI was calculated as the weight in kg divided by the square of the height and using the standard for Asians. Blood pressure was determined on the right arm of the sitting subject after a rest of at least 15 minutes, using a Riester mercury sphygmomanometer. Blood pressure was taken from the mean of two measurements performed by different nurses. Systolic pressure was based on the first Korotkoff sound, whilst diastolic pressure was based on the fifth Korotkoff sound. In case the determinations differed by more than 10 mm Hg, the blood pressure measurement was repeated by a third nurse, and only those measurements were used that differed by <10 mm Hg. Pulse rate was determined after blood pressure measurements, by counting the pulsation of the radial artery for 1 minute, after the subject had rested for at least 15 minutes. The pulse rate was counted twice and the mean of the results was taken.

Analytic procedures

After the subjects had fasted for 12 hours, a 10 mL venous blood sample was collected from each subject for laboratory investigations, viz. fasting blood glucose, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, creatinine, SGOT, SGPT, bilirubin, total protein, albumin, estradiol and VCAM-1. LDL cholesterol and estradiol were determined using reagents from Roche Diagnostics. Assessment of VCAM-1 concentration was by means of sandwich enzyme linked immunosorbent assay (ELISA), using reagents produced by R&D systems, catalog no. DVCOO, lot no. 279922, date of expiry 20 April 2011. The coefficient of variation (CV) of LDL cholesterol assessment was 1.2% between day and 1.7% within run,

For estradiol determination, the CV was 1.7% between day and 1.8% within run, while the CV for VCAM-1 was 2.1% between day and 2.1% within run.

Statistical analysis

The normality of data distribution was determined by using the Kolmogorov-Smirnov test. Data on demographic characteristics, physical measurements, and laboratory tests were presented as descriptive statistics, using the mean and standard deviation for normally distributed data or normalized data after logarithmic transformation. Age of menarche and estradiol concentration were presented as median and interquartile range, because the data were non-normally distributed even after transformation. Pearson correlation tests were used to determine the association between VCAM-1 and several traditional factors such as age, duration of menopause, systolic and diastolic blood pressure, BMI, total cholesterol concentration, HDL and LDL cholesterol, triglycerides, fasting glucose, and estradiol concentration A multiple linear multiple regression analysis was used to determine the most influential factor on VCAM-1 concentration. The level of significance used was 0.05.

Ethical clearance

The study protocol was approved by the Research Ethics Committee, Faculty of Medicine, Trisakti University. All candidate study subjects willing to participate were given information on the aim and benefits of the study, before signing informed consent.

RESULTS

At the start of the study there were 200 subjects who agreed to participate. Eighteen subjects did not meet the inclusion and exclusion criteria, among whom 14 subjects had fasting glucose concentrations of more than 126 mg/dL and 4 subjects were hypertensive, thus resulting in 182 subjects meeting the criteria.

Table. 1 Demographic, physical, and laboratoratory characteristics of the study subjects (n=182)

Characteristic	n (%)
Age (years) #	53.5 ± 3.5
<50	27 (15)
50–54	81 (44)
55 – 60	74 (41)
Age at menarche (years) #	14.3 ± 1.7
<15	146 (80)
=15	36 (20)
Age at menopause (years) #	49.1 ± 3.3
<50	97 (53)
50–54	79 (43)
55–60	6 (6)
Duration of menopause (years)*	4 (3 – 6)
<5	101 (55)
5–10	81 (45)
Education	
No formal education	17 (9)
Did not finish primary school	17 (9)
Primary school	90 (50)
Junior high school	36 (20)
Senior high school	18 (10)
Tertiary education	4 (2)
Employment status	
Employed	84 (46)
Unemployed	98 (54)
Abdominal circumference (cm)#	83.9 ± 10.4
Hip circumference (cm)#	96.9 ± 8.3
Weight (kg)#	59.4 ± 11.3
Height (cm)#	148.9 ± 5.2
Body mass index (kg/m^2)#	26.7 ± 4.7
<25	65 (35.5)
25 – 29.99	79 (43.2)
>30	38 (20.8)
Systolic pressure (mmHg)	124.8 ± 21.3
Diastolic pressure (mmHg)	78.6 ± 12.6
Pulse rate (per minute)	77.9 ± 6.5
Laboratory tests	
Estradiol (pg/mL)*	5.1 (5 – 8.9)
SGOT (U/L)	20.7 ± 5.2
SGPT(U/L)	13.8 ± 7.3
Creatinine (g/dL)	0.65 ± 0.15
Total bilirubin (mg/dL)	0.47 ± 0.20
Protein (g/dL)	7.5 ± 0.5
Albumin (g/dL)	4.4 ± 0.3
Fasting glucose (mg/dL)	87.8 ± 12.7
Total cholesterol (mg/dL)	207.0 ± 36.6
HDL cholesterol (mg/dL)	57.2 ± 12.2
LDL cholesterol (mg/dL)	129.7 ± 33.6
Triglycerides (mg/dL)	115.2 ± 65.5
VCAM-1 (ng/mL)	847.2 ± 275.4

Mean ± SD, * values are median (lowest and highest quartiles). SGOT: serum glutamic oxaloacetic acid transaminase, SGPT: serum glutamic pyruvic transaminase, HDL cholesterol: high density lipoprotein cholesterol, LDL cholesterol: low density lipoprotein cholesterol, VCAM-1: vascular cell adhesion molecule-1

Table 2. Correlation test for several factors affecting VCAM-1 concentration

Factor	VCAM-1	
	r	p
Age at menopause	0.007	0.930
Duration of menopause	0.091	0.220
Abdominal circumference	-0.061	0.417
Systolic blood pressure	0.061	0.414
Diastolic blood pressure	0.053	0.473
BMI	-0.109	0.142
Estradiol	0.111	0.134
HDL cholesterol	0.227	0.069
LDL cholesterol	-0.258	0.001*
Triglycerides	-0.056	0.449
Fasting glucose	0.015	0.843

BMI=body mass index; HDL=high density lipoprotein; LDL=low density lipoprotein

The mean age of the subjects was 53.5 ± 3.5 years, with the majority (44%) between 50 and 54 years of age. Mean age at menarche was 14.3 ± 1.7 years, and 146 subjects (80%) had menarche at the age of less than 15 years. Mean age at menopause was 49.1 ± 3.3 years and 97 subjects (53%) had menopause at the age of less than 50 years. Duration of menopause had a median value of 4 years, with the lowest quartile at 3 years and the highest quartile at 6 years. Ninety subjects (50%) had attended primary school, while a similar percentage had no employment. The demographic and physical characteristics are presented in Table 1.

In addition, Table 1 lists the laboratory parameters of the subjects. Estradiol concentrations had a median of 5.1 pg/mL with the lowest quartile being 5 pg/mL and the highest quartile 8.9 pg/mL. The concentrations of SGOT, SGPT, creatinine, bilirubin, total protein, albumin, fasting glucose, and the lipid profile were within normal limits. VCAM-1 concentration was found to be 847.2 ± 275.4 ng/mL.

Table 2 presents several factors that could possibly affect VCAM-1 expression, namely fasting glucose, estradiol, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, and other factors, such as age at menopause, duration of menopause, BMI, and blood pressure.

Among the various factors in Table 2 above, duration of menopause, estradiol, BMI, total cholesterol, HDL cholesterol, and LDL cholesterol were theorectically associated with VCAM-1 concentration. These five factors were entered into a multiple regression model. Backward model analysis showed that the factor influencing VCAM-1 expression was LDL cholesterol concentration (beta coefficient=0.253; p=0.001) (Table 3).

Tabel 3. Factors assocaiated with VCAM-1 concentration

Factors	Beta coefficient (B)	p
Duration of menopause	0.105	0,152
BMI	-0.010	0.898
Estradiol	0.071	0.349
HDL cholesterol HDL	-0.113	0.157
LDL cholesterol LDL	-0.253	0.001*

*p <0.05 significant

DISCUSSION

Among the five factors analyzed in the present study, only LDL cholesterol had an influence on VCAM-1 expression, with a beta coefficient of –0.253 and p=0.001. The role of LDL cholesterol in the atherosclerotic process lies among others in the formation of foam cells. LDL cholesterol has five subtypes on the basis of molecular size. Small LDL cholesterol molecules are more atherogenic, because they can easily enter the vascular endothelium. The subtypes that are considered to be the most atherogenic are subtypes 4 and 5.[18,19] LDL cholesterol entering the vascular endothelium easily become oxidized LDL cholesterol, which are ultimately phagocytosed by macrophages, which turn into foam cells. Oxidized LDL cholesterol is also one of the factors affecting the decrease in vascular smooth muscle cells through apoptosis in atherosclerotic plaques, secretion of metalloproteinases and other connective tissue enzymes, resulting in a reduction in collagenous tissue, thinning of the atherosclerotic capsule, ultimately making the atherosclerotic plaques vulnerable to rupture.[20-22] The results of the present study are similar with those of the study by Bossowska et al,[23] indicating that the concentration of adhesion molecules (ICAM-1, VCAM-1, sE-selectin) is correlated with traditional risk factors for CVD, such as systolic blood pressure, diastolic blood pressure, and concentration of lipids in the blood.[23]

The mean HDL cholesterol concentration in the present study was 57.2 ±12.2 mg/dL, falling within the reference range of 35–80 mg/dL.[24] HDL cholesterol is essential for lipid transport from the peripheral circulation to the liver, thus decreasing peripheral accumulation of lipids. HDL and LDL cholesterols function synergistically in maintaining cholesterol balance. Reduction of HDL cholesterol is an atherogenic risk factor, whereas an increase in HDL cholesterol concentration acts as a protective factor. Multiple regression analysis indicates that HDL cholesterol is not an influencing factor in VCAM-1 expression, with a beta coeffcient of –0.113 and p=0.157. This differs from the results of a cross-sectional study by Demerath et al.[25] on 592 healthy male and female subjects 18-82 years old, demonstrating an independent association between the concentration of adhesion molecules and HDL cholesterol, particularly in smokers.[25] Demerath's study subjects were in the age range of 18-82 years and were differentiated into smokers and nonsmokers, whereas in the present study all subjects between 47 and 60 years old and did not include any smokers.

In our study, HDL cholesterol was not correlated with VCAM-1. Our result is different from that of a study in Chechia involving both obese and lean postmenopausal women with mean age of 49.9 ± 2.9 years, indicating that serum soluble VCAM-1 concentration is positively correlated with HDL-cholesterol levels.[26] Estradiol concentration in this study had a median of 5.1 pg/mL with the lowest quartile = 5 pg/mL and the highest quartile = 8.9 pg/mL. Estradiol concentration in postmenopausal women had a reference value of <130 pg/mL.[27] All subjects in this study had estradiol concentrations that were below reference value. A study conducted by Ausmanas et al.[27] on 1020 postmenopausal women, comprising 9 Asian ethnic groups, reported that estradiol concentrations varied with ethnic group, the lowest being 13.6 pg/mL in Chinese and the highest 29.1 pg/mL in Vietnamese. The estradiol concentrations in these Asian postmenopausal women had a mean of 20.2 pg/mL (SD = 34.0 pg/mL) and a median of 12.26 pg/mL. In abovementioned study, the estradiol concentration in Indonesian women was 19.6 pg/mL. Ausmanas et al.[28] also confirmed menopausal status by determination of follicle stimulating hormone (FSH) and luteinizing hormone (LH) concentrations. In this connection, FSH concentration was better than estradiol concentration for confirmation of menopause, since FSH is not influenced by

age (p=0.852), in contrast to estradiol (p=0.001).[28] The increased risk for cardiovascular disease in postmenopausal women, such as the subjects of this study, may be attributed to the reduction in estrogen concentrations as one of the causal factors.[6,29]

The American Heart Association has formulated strategies for early detection of CVD in high risk groups, including the use of inflammatory markers,[20,21] as atherosclerosis is thought to be an inflammatory process. Among the various inflammatory markers for primary prevention of CVD,[2,21,28] the one used in this study is VCAM-1, which is both an inflammatory marker and an adhesion molecule.[13,30] Inflammation in atherosclerosis is marked by an infiltration of leukocytes in the vascular endothelium, which is a dynamic organ that in normal circumstances has anticoagulant properties. Injury to the vascular endothelium leads to platelet adhesion and aggregation, ultimately resulting in thrombus formation. Other responses include the mediation of leukocyte migration into the endothelium, and it is this pathological process that is thought to be the initiating event of atherosclerosis.[31-33] In leukocyte migration there are several useful markers, such as intercellular adhesion molecule-1 (ICAM-1), endothelial leukocyte adhesion molecule-1 (ELAM-1), and VCAM-1. VCAM-1 is an adhesion molecule of the IgG superfamily and interacts with its counterreceptor very late appearing antigen 4 (VLA-4), a â1 integrin expressed on the surface of monocytes and lymphocytes.[25,32,34] In this study estradiol concentration was not significantly correlated with VCAM-1 concentration. The results of regression analysis also showed a beta coefficient of 0.071 and a p value of 0.349. Therefore in this study the estradiol concentration did not play a role in VCAM-1 expression. This is in contrast with an in vivo study in rabbits conducted by Nathan et al.[34] to determine the role of estradiol in VCAM-1 expression. The results of the study by Nathan et al.[34] indicated that there were gender differences in monocyte adhesion to vascular endothelial cells and transendothelial migration after induction of hypercholesterolemia in male and female rabbits. The investigators concluded that estradiol presumably inhibits monocyte adhesion and ultimately VCAM-1 expression.[34] The differences in the results of the present study and those of Nathan et al. [34] may be due to the fact that inhibition of VCAM-1 expression presumably constitutes a multifactorial process involving other factors in addition to estrogens. At present the precise mechanisms by which estrogens may protect vascular endothelium from atherosclerosis are as yet not known with certainty.[13,33,34]

Another presumed risk factor for CVD in postmenopausal women is the duration of menopause. In the present study the median duration of menopause in the study subjects was 4 years, with the lowest and highest quartiles being 3 and 6 years, respectively. The majority of the subjects had a duration of menopause of less than 5 years (55%). Bivariate analysis showed that duration of menopause was not correlated with VCAM-1 concentration, while similarly regression analysis showed that duration of menopause was not a factor playing a role in VCAM-1 expression, with a beta coefficient of 0.100 and a p value of 0.175. These results were consistent with those of Women Health Initiative (WHI) study,[6] which indicated that CVD prevalence was not increased with duration of menopause. The WHI study showed a peak prevalence of less than two years and hazard ratio of 1.12, while for durations of 2-5 years and >5 years, the hazard ratios were 1.05 and 0.83, respectively.[6] This WHI study are similar to those of a study conducted by Rossouw et al,[35] showing that the risk of CVD was not influenced by age and duration of menopause.[35]

BMI is another factor presumed to effect a rise in CVD prevalence in postmenopausal women, in whom decreased estrogen concentrations result in altered lipid

metabolism and distribution, from a gynoid type into an android type. With advancing age, the metabolism of the body decreases, such that body weight tends to increase, leading to increased BMI. In the present study, mean BMI was 26.7 ± 4.7 kg/m^2, thus falling into the obese category, and BMI was not correlated with VCAM-1 concentration. The results of a linear regression analysis also showed that BMI did not influence expression of VCAM-1, with a beta coefficient of –0.010 and p=0.898. These results are contrary to those of a study by Bossowska et al,[23] demonstrating a correlation of ICAM-1 and VCAM-1 concentrations with BMI. These conflicting results may have been due to different characteristics of the study subjects. The study by Bossowska et al [23] used male and female subjects with mean age of 60 years with past myocardial infarction, whereas the present study involved healthy postmenopausal women as subjects. In addition, 65% of the subjects of the present study had BMI values of more than 25 kg/m^2, thus resulting in inadequate variation in BMI among the study subjects to represent the normal, obese I and obese II categories.[36]

One of the limitations of the present study is that menopause was confirmed by past history (anamnesis) and estradiol concentrations in the blood, and not by follicle-stimulating hormone (FSH) concentration. Another limitation was that the study used a cross-sectional design, such that it yielded only the factors affecting VCAM-1 concentration, but could not demonstrate a casuse-and-effect relationship. Therefore it is necessary to conduct experimental studies to determine whether or not LDL cholesterol and VCAM-1 concentrations are involved in a cause-and-effect relationship.

CONCLUSION

The traditional risk factor with the greatest influence on VCAM-1 expression in postmenopausal women is LDL cholesterol concentrration.

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