ORIGINAL ARTICLE

Evaluation of Endothelial Function in Pre-Menopausal Women with Coronary Arterial Disease

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Abstract

Background: The endothelium plays an important vascular regulatory function. Its dysfunction is an early marker of cardiovascular risk. However, there are few studies in our community that assess endothelial function in pre-menopausal women.

Objective: To assess endothelial function in pre-menopausal women in the presence or absence of coronary artery disease, using a biophysical method (carotid intima media thickness) and a biochemical method (serum levels of hsCRP).

Methods: Cross-sectional study that evaluated carotid intima-media thickness and serum levels of hsCRP of 31 pre-menopausal women undergoing coronary angiography at the Hemodynamics Service of Hospital Universitário da Universidade Federal do Maranhão from March 2012 to July 2013. The data were sent to statistical analysis and a statistical significance level of 5% was considered.

Results: The sample was divided into two groups according to the presence of coronary artery disease (CAD): CAD group (n = 13) and group without CAD (n = 18). The average ages for the groups were 57.92 ± 5.17 and 51.72 ± 4.63 years, respectively (p = 0.001). CIMT was abnormal in 29.03% in the general population. Carotid intima-media thickness was 1.55 ± 0.78 mm in the general group, 1.92 ± 0.94 mm in the CAD group and 1.18 ± 0.71 mm in the group without CAD (p = 0.001). CAD patients had predominance of abnormal CMIT compared those without CAD: 36.46% vs. 22.22%, respectively. There was a sensitivity of 38%, specificity of 77% with a positive predictive value of 0.55 and a negative predictive value of 0.63 with likelihood ratio of 1.73. Patients with abnormal CIMT presented higher levels of hsCRP, but without statistical significance. CAD patients had higher levels of hsCRP, but without statistical significance.

Conclusion: In the population studied, assessment of endothelial function using the CIMT method showed higher sensitivity and specificity for the diagnosis of CAD compared to the measurement of hsCRP levels in menopausal women. (Int J Cardiovasc Sci. 2017;30(3):227-234)

Keywords: Coronary Artery Disease; Endothelium/dysfunction; Women; Premenopause; Atherosclerosis; Cineangiography.

Introduction

Cardiovascular diseases (CVD) are the leading cause of overall mortality in women, accounting for 34% of total deaths in 2011. In Brazil, it is the primary cause of death in women over 60, accounting for 39% of deaths in this age group in 2011.[1]

The incidence of CVD significantly increases in women after menopause. Hormonal changes that occur during this period and the vascular and blood effects associated are recognized as participants in the onset and progression of CVD and therefore relate to this increased risk of illness.[2]

The endothelium is the main regulator of vascular homeostasis, as it does not only perform its barrier function, but also plays a crucial role in the paracrine control of vascular smooth muscle and in the response

to current stimuli, coagulation, leukocyte adhesion and vascular proliferation.[3,4] It is one of the main regulators of vascular biology. Its dysfunction plays a central role in the pathophysiology of atherosclerosis development.[5,6]

Endothelial dysfunction is the endothelial inability to appropriately react to stimuli, especially with respect to its function on the vascular tone. Such dysfunction is one of the earlier markers for the risk of developing CVD,[7,8] possibly contributing to increased cardiovascular morbidity in the menopausal period.

The study of endothelial function is done mainly through non-invasive biophysical and biochemical methods. Among the biophysical methods, ultrasound measurement of the carotid intima-media thickness (CIMT) is a strong predictor of future cardiovascular events,[9,10] serving to monitor the progress or regress of atherosclerotic lesions,[11] and is useful in identifying subclinical vascular diseases,[12] being an excellent factor in the assessment of cardiovascular risk.

Among the biochemical markers, platelet activation markers and inflammation, especially ultrasensitive C-reactive protein (hsCRP), also prove useful in the assessment of endothelial function.[13] HsCRP is an acute phase protein synthesized by the liver in a systemic response to an inflammatory condition. Some studies have shown that this is a useful marker to evaluate the presence of vascular inflammatory processes, being related to the development of vascular adverse events and CVD.[14,15]

This study aimed to evaluate endothelial function in pre-menopausal women in the presence or absence of coronary artery disease, using CIMT measurements and hsCRP levels.

Methods

Cross-sectional analytical study that evaluated 31 pre-menopausal women aged between 40 and 65, who underwent coronary angiography at Hospital Universitário da Universidade Federal do Maranhão (HUUFMA) between March 2012 and July 2013, and agreed to participate in the study after signing the Informed Consent Form.

During the study period, 2046 coronary angiography procedures were performed, of which 815 were in women. Of these, 447 were in the menopausal period, and 368 did not meet the inclusion criteria. Of the 79 women able to participate in the study, 48 have abandoned the research protocol for several reasons, and were not considered in the sample, which was set at 31 participants who completed all the assessments.

The study did not include pregnant patients on statins, those undergoing coronary angioplasty or with coronary stents, or those with history of acute myocardial infarction. Based on the results of coronary angiography, patients with coronary artery disease (CAD) were identified. The sample was then divided into two groups: group I, with CAD (n = 13); group II, without CAD (n = 18).

For sociodemographic characteristics, we collected their age, self-reported skin color, education and household income. All data collected from patients were recorded on standardized protocol forms for this study.

To measure the carotid intima-media thickness (CIMT), we used the method first described by Pignoli et al.[16] With the patient in the supine position, the neck was exposed, inclined and turned to the opposite side to improve visualization of the vessels. Placing the transducer, the carotid wall is then viewed, and the thickness measured by the distance between two well-defined echogenic lines separated by a discrete anechoic strip.[16] Any values greater than 0.9 mm[17] were considered abnormal.

CIMT measurements were taken by an experienced sonographer, blind to the results of coronary angiographies, who used a two-dimensional ultrasound device with pulsed Doppler, color flow mapping and a linear transducer operating at 7.5 MHz (Philips Ultrasound®, HD7, software revision 2.0.1, Bothell - USA).

HsCRP measurements were taken at the HU-UFMA laboratory, and the samples were collected after the patients went through a 12-hour fasting.

The cutoff values for hsCRP normality were low cardiovascular risk (< 1 mg/L), moderate risk (1 to 3 mg/L) and high risk (> 3 mg/L).[18]

Statistical analysis

Statistical analyses were performed using the Data Analysis and Statistical Software (STATA®) version 12.0 (Stata Corp., College Station, United States). Categorical variables were described by frequencies and percentages and the numeric variables, by mean ± standard deviation. To investigate the association of categorical data, Fisher's exact test and the chi-square test were used. Normality was verified by the Shapiro-Wilk test. Mann-Whitney test or unpaired t-test for independent samples was used to identify statistically significant differences between groups. All analyses considered a statistical significance level of 5% ($p < 0,05$).

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This manuscript is part of a set of projects from a larger cross-sectional study called "Disfunção Endotelial e Avaliação do Risco Cardiovascular em Mulheres Climatéricas" [Endothelial Dysfunction and Cardiovascular Risk Assessment in Pre-Menopausal Women," approved by the institution's ethics committee under opinion no. 182/11, following Resolution 196/96 and other complementary resolutions issued by the Brazilian National Council of Health (CNS/MS).

Results

In this study, 31 pre-menopausal women met the inclusion criteria and participated in all of its stages. The average age of the entire group was 54.32 ± 5.70. The mean age of the groups with and without CHD was 57.92 ± 5.17 and 51.72 ± 4.63, respectively, statistical significance (p = 0.001).

There was a predominance of brown women (70.97%), with monthly household income lower than two minimum wages (58.06%) and education of more than 8 years (51.61%) (Table 1).

CIMT was abnormal in 29.03% in the general population. The intima-media thickness was 1.55 ± 0.78 mm in the general group, 1.92 ± 0.94 mm in the CAD group and 1.18 ± 0.71 mm in the group without CAD (p = 0.001) (Table 2). CAD patients had predominance of abnormal CMIT compared to those without CAD: 36.46% vs. 22.22%, respectively (Table 1).

Patients with abnormal EMI had higher levels of hsCRP, but with no statistical significance (Table 3).

CAD patients had higher levels of hsCRP, but with no statistical significance (Table 3).

Discussion

Atherosclerotic disease is the leading cause of morbidity and mortality in Western communities.[19] Its incidence in women is increasing along with changes in lifestyle.[20]

Another peculiarity related to CAD affecting women is that the onset of the disease is typically late, especially after menopause. This characteristic requires reliable diagnostic tools that may indicate early dysfunction and risk factors for cardiovascular disease.

A study conducted in Europe in 2008 by Allender et al.[21] showed that coronary disease was responsible for 23% of deaths in women. Several studies have demonstrated the increased incidence of CAD with increasing age and menopause. Tremollieres et al. (1999) found a 36% prevalence of CAD in most women after menopause.[22]

Endothelium dysfunction is extremely important in the pathophysiology of atherosclerosis.[23] The main methods currently available for assessing endothelial function consist in measuring endothelial response to physical or pharmacological stimuli.

Platelet activation markers and inflammation, especially ultrasensitive C-reactive protein (hsCRP), also proved useful in the evaluation of endothelial health.[24]

CIMT has been considered a useful, low-cost technique for early detection of atherosclerosis and as a predictor of risk of cardiovascular events.[25]

Kablak-Ziembicka et al.,[26] in a study involving 558 patients of both genders, including 120 women, with a mean age of 58.8 ± 9.2, showed that there is an association of greater CIMT abnormality in patients with angiographically confirmed CAD than in patients who had normal coronary arteries.

Several studies have suggested a correlation between the levels of hsCRP and CMIT.[28-31] Trinidad et al.,[27] in a study conducted at Universidade Estadual do Rio de Janeiro with 116 hypertensive women aged 40 to 65, showed hsCRP correlated to CIMT. Similarly, the findings of a work developed by Blackburn et al. with 1,051 individuals with dyslipidemia showed correlation between hsCRP and CIMT.[28] The studies of Wang et al.[29] and Sitzer et al.[30] also showed correlations between these variables.

Kawamoto et al.,[31] in a study of 440 patients of both genders, including 201 women aged 75±10, found that hsCRP levels were associated with increased CIMT.

Parildar et al.,[32] found a positive and significant correlation between CIMT and hsCRP in a study involving 110 pre-diabetic patients and 76 healthy patients with mean age of 51.1 ± 9.9, with a percentage of female patients of 68.1%.

Besides this, Amer et al.,[33] in a case-control study involving elderly hypertensive patients, found that CIMT had a positive and significant correlation with hsCRP levels.

On the other hand, the association between CIMT and hsCRP levels were not significant in some studies. Folsom et al.,[34] examined the association of hsCRP with atherosclerotic disease markers in a study conducted at the University of Minnesota in 2001, involving 875 men and 948 women, and found no significant correlation between these variables.

Table 1 – Sociodemographic and health characteristics of pre-menopausal women – HUUFMA. São Luís – MA, 2013							
Variables	**General**		**CAD**				**P-value**
			Present		**Absent**		
	N	**%**	**n**	**%**	**N**	**%**	
Education							0.833[B]
≤ 8 years	15	48.39	6	46.15	9	50.00	
> 8 years	16	51.61	7	53.85	9	50.00	
Skin color							0.443[A]
White	6	19.35	3	23.08	3	16.67	
Black	3	9.68	0	-	3	9.68	
Brown skin	22	70.97	10	76.92	12	66.67	
Household income							0.157[A]
< 1 minimum wage	6	19.35	4	30.77	2	11.11	
Between 1 and 2 minimum wages	12	38.71	6	46.15	6	33.33	
> 2 minimum wages	13	41.94	3	23.08	10	55.56	
Hypertension							0.880[A]
Absent	10	32.26	4	30.77	6	33.33	
Present	21	67.74	9	69.23	12	66.67	
Diabetes mellitus							0.151[A]
Absent	27	87.10	10	76.92	17	94.44	
Present	4	12.90	3	23.08	1	5.56	
High cholesterol							0.155[A]
Absent	22	70.97	11	84.63	11	61.11	
Present	9	29.03	2	15.38	7	38.89	
CVD							0.955[A]
Absent	24	77.42	10	76.92	14	77.78	
Present	7	22.58	3	23.08	4	22.22	
Alcoholism							0.621[A]
Absent	27	87.10	12	92.31	15	83.33	
Present	4	12.90	1	7.69	3	16.67	
Current smoking							0.232[A]
No	30	96.77	12	92.31	18	100	
Yes	1	3.23	1	7.69	0	0	

To be continued

Continuation

Former smoker							0.331[A]
No	22	75.86	8	66.67	14	82.35	
Yes	7	24.14	4	33.33	3	17.65	
Physical activity							0.641[A]
Absent	20	64.52	9	69.23	11	61.11	
Present	11	35.48	4	30.77	7	38.89	
Menopause							0.260[A]
Absent	8	25.81	2	15.38	6	33.33	
Present	23	74.19	11	84.62	12	66.67	
Hormone replacement							0.268[A]
No	26	89.66	11	100	15	83.33	
Yes	3	10.34	-	-	3	16.67	
CIMT							0.433[A]
Normal	22	70.97	8	61.54	14	77.78	
Abnormal	9	29.03	5	36.46	4	22.22	
Age (mean±standard deviation)	54.32 ± 5.70		57.92 ± 5.17		51.72 ± 4.63		0.001[#]
SBP (median; Q3-Q1)	135 (170 – 120)		135 (180 – 125)		135 (160 – 120)		0.400[*]
DBP (mean ± standard deviation)	84.74 ± 11.27		84.23 ± 11.87		85.11 ± 11.15		0.834[#]

[#] t-test; [*] Mann-Whitney test; [A] Fisher exact test; [B] Qui-square test; Q3-Q1 - quartile 3 and quartile 1.

Table 2 – Association of CIMT with CAD in pre-menopausal women – HUUFMA. São Luís – MA, 2013

Characteristic	General	CAD		P-value
		Present	Absent	
	Mean ± SD	Mean ± SD	Mean ± SD	
CIMT	1.55 ± 0.78	1.92 ± 0.94	1.8 ± 0.71	0.001[#]

HUUFMA: Hospital Universitário da Universidade Federal do Maranhão; CIMT: carotid intima-media thickness; CAD: coronary artery disease; [#] t-test.

Similarly, the results of the study by Hak et al.[35] conducted in 186 healthy middle-aged women selected from the general population, indicated that hsCRP is not significantly associated with CIMT.

Jie Cao J. et al,[36] conducted a cohort of 5417 participants to evaluate the correlation between hsCRP and CIMT in elderly patients with high risk of stroke. They concluded that high levels of hsCRP represent an independent risk factor for stroke, not correlating with the severity of atherosclerotic plaque as measured by CIMT.

The literature considers the association between higher levels of hsCRP with risk of cardiovascular events in the general population to be well-established. In a meta-analysis of 160,309 patients published in 2009

Table 3 – Association of HsCRP with CIMT and CAD in pre-menopausal women – HUUFMA. São Luís – MA, 2013		
Variables	**HsCRP**	
	mg/L	**p-value**
CIMT		0.1282#
Normal	3.22 ± 3.76	
Abnormal	4.66 ± 4.35	
CAD		0.836#
Absent	3.80 ± 3.10	
Present	4.00 ± 4.40	

HUUFMA: Hospital Universitário da Universidade Federal do Maranhão; HsCRP: ultrasensitive C-reactive protein; CIMT: carotid intima-media thickness; CAD: coronary artery disease; # t-test.

by the Emerging Risk Factors Collaboration group, hsCRP levels were linearly associated with the presence of several cardiovascular risk factors and inflammatory markers and were strongly associated with the risk of ischemic vascular diseases.[14]

In this study, although serum levels of hsCRP are higher among women with CAD, no relationship was found between these variables. It should be considered that, in both groups, the patients already had cardiovascular risk factors, which may have led to the convergence of hsCRP values.

Several studies have shown baseline levels of hsCRP as independent predictors for coronary artery disease.[15,38] A prospective case-control study conducted by Boekholdt et al.[37] with 25,663 men and women aged between 45 and 79, who were part of the EPIC-Norfolk study, found that hsCRP levels were among the main predictors of coronary artery disease and mortality.

A prospective case-control study conducted by Ridker et al.[39] evaluated, for three years, the levels of inflammatory markers of 28,263 apparently healthy women in post-menopause. The study also found cardiovascular events during the study period. HsCRP levels proved to be the most important independent predictors of risk of cardiovascular events in this group.[15]

The disagreements between the medical literature available and some of our results can be explained by the sample of limited size, resulting from losses during the study, and the fact that our sample is composed solely of patients with clinical indication for coronary angiography, having different characteristics compared to the general population of pre-menopausal women.

Clinical implications

This study fills a gap of few national studies assessing endothelial function in pre-menopausal patients.

Study limitations

The main limination of this study was a non-probabilistic sample limited to a relatively small number of individuals, making studies with larger sample sizes necessary.

Conclusion

In the population studied, assessment of endothelial function using the CIMT method showed higher sensitivity and specificity for the diagnosis of CAD compared to the measurement of hsCRP levels in pre-menopausal women.

Author contributions

Conception and design of the research: Farias WKS, Figueiredo Neto JA. Acquisition of data: Farias WKS, Melo JB, Fonseca EJNC, Pontes LP, Andrade MVG. Analysis and interpretation of the data: Farias WKS, Rocha TPO, Fonseca EJNC, Fernandes DR.

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