

Assessment of Endothelial Dysfunction by Brachial Artery Flow-Mediated Dilatation in Postmenopausal Women at Low Risk for Cardiovascular Disease

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ABSTRACT

Objective: The objective is to compare endothelial dysfunction measured by brachial artery flow-mediated dilation (BAFMD) in nonobese, nondiabetic post-menopausal women with their age-matched menstruating controls and to identify the correlation of BAFMD with Framingham risk score (FRS) and with the individual parameters of FRS in low-risk women. **Methods:** This study was done in the department of Obstetrics and Gynaecology, Chandigarh, India, for 1 year. Fifty postmenopausal and 50 menstruating females aged 45–55 years who were nondiabetic and nonobese and were low risk according to FRS were selected as cases and controls, respectively. All cases and controls were age-matched. The diameter of the brachial artery and the blood flow in it was measured at rest. Ischemia was produced and released after 5 min. The maximum blood flow velocity diameter of the brachial artery was measured. After 10 min of reactive hyperemia, 400 µg of sublingual nitrate was given, and vasodilatation mediated by nitroglycerine was subsequently measured. **Results:** Menopause did not have any significant effect on the endothelial dysfunction as measured by the brachial artery flow-mediated dilatation ($P = 0.74$) but did influence vascular smooth muscle as measured by nitroglycerine-mediated dilatation ($P = 0.028$). A significant correlation was found between flow-mediated dilatation with FRS helps us conclude that flow-mediated dilatation is a reliable tool to estimate the cardiovascular risk ($P < 0.001$). A strong correlation was found between nitroglycerine-mediated dilatation and flow-mediated dilatation, demonstrating that both endothelial dysfunction and vascular smooth muscle are interrelated ($P < 0.001$). **Conclusion:** Menopause did not affect endothelial function, but it has a significant effect on vascular smooth muscle function. To know the effect of longer duration of menopause on vascular function in elderly women further studies with large number of postmenopausal women of different duration of menopause, may be needed.

KEYWORDS: Brachial artery flow-mediated dilatation, cardiovascular disease, endothelial dysfunction, Framingham risk score, menopause, nitroglycerine-mediated dilatation

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INTRODUCTION

Menopausal health demands priority in the Indian scenario due to increase in life expectancy and growing population of menopausal women. Menopause is defined by the World Health Organization as the permanent cessation of menses because of the loss of ovarian follicular function or surgical removal of

ovary.^[1] The median age of menopause is estimated to be 50–52.76 year.^[1] It is well known that cardiovascular

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risk is higher in postmenopausal women than in premenopausal women, but it is unclear how much of the elevated risk is related to aging, menopause itself, or the presence of other confounding factors. Endothelial dysfunction is one of the most important predictors for determining early atherosclerotic risks as it precedes overt vascular disease by years and may itself be a potentially modifiable cardiovascular disease (CVD) risk factor.

Although no gold standard for the measurement of endothelial function exists, the measurement of flow-mediated dilation (FMD) in the brachial artery, assessed with Doppler ultrasonography, is the most studied method and seems most promising for clinical application. Trials have concluded that in apparently healthy individuals with relatively few risk factors, brachial artery flow-mediated dilation (BAFMD) provides distinct, independent information about the complex atherosclerotic process. Teragawa *et al.* determined the cut-off value for FMD for detecting the presence of coronary artery disease as 6%, with a sensitivity of 0.93 and a specificity of 0.88.^[2] Various studies highlighted that FMD was impaired in the diabetics, obese, and patients falling either in the 2nd or 3rd tertiles of the (Framingham risk score [FRS]) range.^[3-5]

Keeping in mind all these important parameters affecting endothelial function, the objective of this study was to assess the endothelial dysfunction by BAFMD in postmenopausal and menstruating nondiabetic, nonobese, females of the same age group coming under low risk by Framingham risk scoring system. A comparison between the two groups was also made to assess the effect of menopause on endothelial dysfunction.

PATIENTS AND METHODS

Study design

A case-control study was conducted in Departments of Obstetrics and Gynaecology and Cardiology, Post Graduate Institute of Medical Education and Research, Chandigarh, for 1 year.

Patient population

We identified women between 45 and 55 years of age with the absence of menses for 1 year. Controls included the women of the same age group who had not attained menopause. All cases and controls were taken consecutively. To detect a statistically significant difference of approximately 4%–8% between cases and controls, 50 women were recruited in each group with a power of 90% and confidence interval of 95%.

Procedure

The participants were positioned in a supine position, in a quiet room, with constant temperature, the arm placed in a comfortable position for assessing the brachial artery, and remained under constant conditions for at least 10 min. The patient did not ingest substances that might affect FMD, such as caffeine, high-fat food, and Vitamin C nor smoke, for at least 6 h before the study. The brachial artery was imaged above the antecubital fossa in the longitudinal plane, using a linear array transducer (with frequency 7–12 MHz) attached to a high-quality mainframe ultrasound system (iE33, Philips ultrasound, USA). Initially, the diameter of the brachial artery was determined at rest, and blood flow was estimated by time averaging the pulsed Doppler velocity signal obtained from a mid-artery sample volume. The diameter of the brachial artery was determined [Figure 1a and b]. To decrease the variability of the measurements, the brachial artery diameter was determined by the average derived from multiple diameter measurements along the same segment of the vessel. During image acquisition, anatomic landmarks such as veins or fascial planes were noted to help maintain the same image of the artery throughout the study. Ischemia was produced by inflating a cuff placed at the distal forearm, at a pressure 50 mm Hg greater than the systolic blood pressure (BP), for 5 min. The ischemia cuff was released after 5 min [Figure 1c]. The maximum blood flow velocity was detected by analyzing mid artery pulsed Doppler signal after 15 s of cuff release, while the maximum diameter of the brachial artery will be determined 60 s after release. The brachial artery was continuously monitored from 30 s before to 120 s after ischemia cuff release. Flow-mediated vasodilation was expressed as the change in poststimulus diameter as a percentage of the baseline diameter.

$$\text{FMD} = (\text{Maximum diameter after cuff release} - \text{baseline diameter}) / (\text{baseline diameter}) \times 100.$$

Measurement of Nitroglycerine mediated dilatation (NMD)

After 10 min of reactive hyperemia (time needed to re-establish baseline conditions) a single bolus of 400 µg of sublingual nitro-glycerine (NTG) nitrates was given. Peak vasodilation occurred 3–4 min after nitrate administration.

$$\text{NMD} = (\text{Maximum diameter after nitro-glycerine} - \text{baseline diameter}) / (\text{baseline diameter}) \times 100.$$

The study protocol and the consent procedure were approved by the ethics committee of the institute. The women who participated in the study were informed of the study objectives, and written consent was obtained, documented, and classified.

Statistical analysis

Statistical analysis was done using SPSS version 15 (Statistical Packages for the Social Sciences, Chicago, IL, USA). Quantitative data were presented as mean \pm standard deviation (SD) or median and Interquartile range, as appropriate. For categorical variables, number and percentages were calculated. For the low-risk group, mean \pm SD or median and interquartile range of FMD and NMD were calculated. Normality of data was checked by measures of Kolmogorov–Smirnov tests of normality. For normally distributed data means were compared using independent sample *t*-test and if it was skewed Mann–Whitney test was applied. All calculations were two-sided. A $P < 0.05$ was considered to indicate statistical significance.

RESULTS

Hundred nonobese, nondiabetics, females; 50 menopausal, and 50 menstruating coming under low risk according to FRS were taken as cases and controls, respectively. All cases and controls were age-matched (frequency matching was done). BAFMD and NMD were calculated in all the subjects.

In both case and control groups, 27 women of age group 45–49 years and 23 women of age group 50–55 years were recruited. In both case and control groups, the mean age was 49.40 ± 3.648 years. Among the postmenopausal women, the maximum number of subjects had their last menstrual period 1–2 years ago. Subjects in both the groups were divided according to their Framingham's risk score in three groups. Nine percentage of subjects in both the groups were in the lowest tertile group, 25% in the middle tertile group, 66% in the highest tertile group [Table 1].

The effect of menopause on BAFMD, NMD, FRS, and individual components of FRS was studied. The means of every parameter was calculated in both the groups. A significant difference was found in mean NMD and mean total cholesterol values in between the two groups ($P = 0.028$ and 0.001 , respectively). Although mean FMD (8.3 vs. 7.3) and mean FRS was higher in the postmenopausal group (7.2 vs. 6.6), the difference

was not significant statistically ($P = 0.074$ and 0.143 , respectively). Mean high-density lipoprotein (HDL) cholesterol was lower in postmenopausal women, but the difference was not significant statistically ($P = 0.4$). Mean systolic BP was similar in both the groups < [Table 2].

On studying the various parameters affecting the endothelial function, a statistically significant negative correlation was found between FMD and FRS ($P < 0.001$), FMD and Age ($P < 0.001$) and FMD and duration of menopause ($P < 0.001$) [Table 3].

On studying the effects of FRS, individual components of FRS, and duration of menopause on smooth muscle function, statistically significant negative correlation was

Table 1: Comparison of demographics

Characteristics	Premenopausal (n)	Postmenopausal (n)
Age (years)		
45-49	27	27
50-55	23	23
Duration of menopause (years)		
1-2		36
3-5		11
>5		3
FRS score		
0-3	4	5
4-6	11	14
7-9	35	31

FRS: Framingham risk score

Table 2: Effects of menopause

	Postmenopausal	Premenopausal	P
Mean BAFMD	8.334 \pm 2.47	7.335 \pm 3.01	0.074
Mean NMD	8.222 \pm 2.53	7.120 \pm 2.37	0.028
Mean FRS	7.22 \pm 1.877	6.63 \pm 2.038	0.143
Mean total cholesterol	169.94 \pm 14.578	159.70 \pm 13.080	<0.001
Mean HDL cholesterol	47.50 \pm 5.534	46.67 \pm 6.05	0.480
Mean SBP	121.5 \pm 9.845	121.2 \pm 8.411	No difference

BAFMD: Brachial artery flow-mediated dilation, NMD: Nitroglycerine-mediated dilatation, FRS: Framingham risk score, HDL: High-density lipoprotein, SBP: Systolic blood pressure

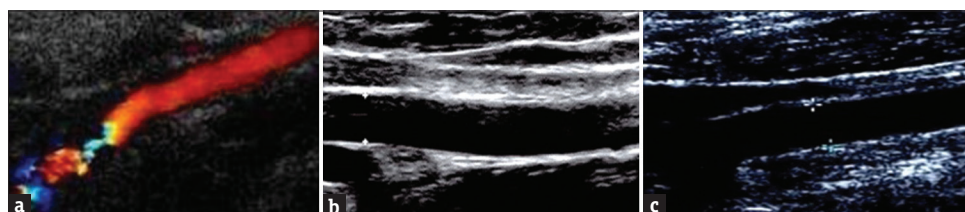


Figure 1: (a) Brachial artery was localised in the ante-cubital fossa with the help of pulsed Doppler signal. (b) Base-line diameter was measured by positioning the callipers on the intimal layer of the vessel lumen. (c) Diameter was measured again after five minutes of cuff inflation

found between NMD and FRS ($P < 0.001$) and NMD and age ($P = 0.05$) [Table 4].

We also found a significant correlation between FMD and NMD ($P < 0.001$), signifying that both endothelial dysfunction and vascular smooth muscle dysfunction are interrelated.

On evaluating the strength of correlation of FMD with FRS, different parameters of FRS, and duration of menopause, FMD was found to have a statistically significant negative correlation with all these parameters [Table 5].

Table 3: Factors affecting endothelial dysfunction

	Correlation with FMD	Coefficient of correlation	P
FRS	Negative	-0.492	<0.001
Age	Negative	-0.321	<0.001
Duration of menopause	Negative	-0.281	0.049
Total cholesterol	Negative	-0.058	0.568
HDL	Positive	0.098	0.333
SBP	Negative	-0.075	0.460

FRS: Framingham risk score, HDL: High-density lipoprotein, FMD: Flow-mediated dilation, SBP: Systolic blood pressure

Table 4: Factors affecting smooth muscle function

	Correlation with NMD	Coefficient of correlation	P
FRS	Negative	-0.445	<0.001
Age	Negative	-0.278	0.05
Duration of menopause	Negative	-0.251	0.082
Total cholesterol	Negative	-0.038	0.706
HDL cholesterol	Positive	0.047	0.643
SBP	Negative	-0.053	0.603

FRS: Framingham risk score, HDL: High-density lipoprotein, NMD: Nitroglycerine-mediated dilatation, SBP: Systolic blood pressure

Table 5: Strength of correlation of flow-mediated dilation and nitroglycerine-mediated dilatation with Framingham risk score, different parameters of Framingham risk score and duration of menopause

	Pearson's correlation coefficient	Strength of correlation	P
FMD and FRS	-0.492	Moderate	<0.001
FMD and age	-0.321	Moderate	<0.001
FMD and duration of menopause	-0.281	Weak	0.049
NMD and FRS	-0.445	Moderate	<0.001
NMD and age	-0.278	Weak	0.05
NMD and duration of menopause	-0.251	Weak	0.082

FMD: Flow-mediated dilatation, FRS: Framingham risk Score, NMD: Nitroglycerine-mediated dilatation

In our study, NMD was found to have a statistically significant negative correlation with FRS and age ($P = 0.001$ and $P = 0.05$, respectively) [Table 5].

DISCUSSION

The present study compared the endothelial dysfunction in nondiabetic, nonobese, postmenopausal women, at low risk for CVD by FRS score of age group 45–55 years, measured by BAFMD with their age-matched menstruating controls. The aim was to find out if menopausal women who are at low-risk FRS have subclinical dysfunction of vascular endothelium or smooth muscle as compared to premenopausal women. We did not find any statistically significant difference in endothelial dysfunction measured by BAFMD in both groups. This made us to conclude that in age-matched women with low-risk FRS menopausal status does not cause endothelial dysfunction. This agreed with the Nurses' Health Study, in which after adjusting for age, smoking status, and other cardiovascular risk factors, the relative risks of cardiovascular events across categories of age at natural menopause (<40, 40–44, 45–49, 50–54, and ≥ 55 years) were 1.53, 1.42, 1.10, 1.00, and 0.95, respectively.^[6,7]

The results of the present study were contrary with the large Framingham study in which there was a significant rise in coronary heart disease (CHD) incidence after menopause. In our study all the women included were age matched compared to the Framingham study where the study group were divided into specific age groups. As smoking was not included in our analysis, this may have affected the results.^[6,8]

Although many other studies conducted in the past had shown a significant difference in risks of cardiovascular events in postmenopausal and premenopausal women, none of them had taken all age-matched low-risk women. Hence, the nonsignificant effect of menopause on endothelial function in our patients was most likely due to all of them being in low-risk and age matched.

However, in our study, NMD was significantly higher in the postmenopausal group. This implies that postmenopausal women have a significantly higher vascular smooth muscle dysfunction despite being at low risk of CVD as per the FRS. Although no similar study showing the effect of menopause could be found in literature, the study carried out by Adams *et al.* showed a reduced vasodilator response to NTG was associated with high cholesterol, cigarette smoking, diabetes mellitus, increasing age, male gender, larger vessel size, and reduced FMD ($P/0.01$ for all), showing significant smooth muscle dysfunction in adults at risk for atherosclerosis.^[9]

A significant correlation between FMD and NMD was also found, and the strength of correlation was strong. This was in accordance with the study carried out in geriatric subjects in Istanbul University, in which they also found a correlation between FMD and NMD ($r = 0.22, P < 0.02$).^[10]

Relation of menopause with FRS and different components of FRS was studied. The significant difference was found only in total cholesterol levels in pre- and post-menopausal women.

FRS and other components of FRS were not found to significantly correlate with menopause. This was partially in agreement with the third French MONICA (Monitoring of trends and determinants in CVD) the study carried out by Agriniera *et al.* in 2009 in which serum total cholesterol and the Framingham 10-year risk of CHD were higher in the postmenopausal women. In our study, there was no significant difference in systolic BP, HDL cholesterol, and FRS in both the groups. Similar results were also seen in the above-mentioned study in which body mass index, BP, fasting glycemia, triglyceride, serum HDL cholesterol, and apolipoprotein A1 levels did not differ according to menopausal status after adjustment for age.^[11]

In our study, FMD was found to have a significant negative correlation with FRS, i.e., as the FRS increases, the FMD decreases. Thus, even when the women have low-risk FRS as the value of score rises the endothelial function gets affected. The strength of correlation was moderate. This agreed with the study carried out by Rossi *et al.*, in which they found a significant correlation between FMD and cardiovascular events. The event rate in 2,264 post-menopausal women aged 54 ± 6 years was studied. The event rate among patients in the lowest tertile of FMD was greater than the combined event rate observed in the other two tertiles. The number of women having cardiovascular risk significantly varied according to tertiles of FMD.^[12]

In our study, we found a significant negative correlation of FMD with age, and the strength of correlation was moderate; however, other components of FRS (total cholesterol, HDL cholesterol, systolic BP, and smoking) were not found to have any significant correlation with FMD. This was in agreement with the large study carried out by Rossi *et al.* in which age, gender, and smoking were independently associated with FMD, but other CVD risk factors were not.^[12]

In our study, we also found a significant negative correlation of NMD with FRS, and the strength of correlation was moderate. This was in agreement with

the study carried out by Kwagyan *et al.*, in which they found a significant correlation between NMD and FRS ($r = -0.31, P = 0.001$).^[13]

In our study, the only parameter of FRS which effected NMD was age, however, the strength of correlation was weak. This was similar to the study carried out in geriatric subjects in Istanbul University, in which they found the only parameter which had a significant effect on NMD was age.^[10]

In the present study a significant negative correlation was also found between FMD and duration of menopause, which showed a significant effect of duration of menopause on cardiovascular risk, however, this strength of correlation was weak. This result is very well supported by various large studies including Nurse's health study^[6] and SWAN studies.^[14]

Strength and limitations of the study

Our primary strength was that it was a case-control study, and the controls were age-matched which minimizes the bias. However, our study had certain limitations. The study sample size was small. We did not keep follow-up of our patients and hence did not have any clinical end points.

CONCLUSION

Our study concluded that the menopausal status does not affect endothelial function in women who are at low risk of CVD, but it affects the vascular smooth muscle function. Furthermore, the correlation of the duration of menopause with FMD and not with NMD made us conclude that smooth muscle dysfunction occurred more immediately after menopause in comparison to endothelial dysfunction as most of the subjects in our study group were in their early menopausal period. However, endothelial dysfunction increased as the duration of menopause increases whereas smooth muscle dysfunction remained relatively constant. This suggests that as the duration of menopause is directly proportional to the risk of cardiovascular event in menopausal women. Our study was not strong enough to assess this clinical end point. The present study was carried out in low-risk women in an age group near menopause. To know the effect of longer duration of menopause on vascular function in elderly women further studies with large number of postmenopausal women, of different duration of menopause may be needed.

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Conflicts of interest

There are no conflicts of interest.

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