Original Research Article

Assessment of endothelial dysfunction by brachial artery flow mediated dilation in postmenopausal women at low risk for cardiovascular disease

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ABSTRACT

Introduction: The increase in life expectancy has resulted in increased population of menopausal women hence menopausal health has become a priority in Indian scenario. World Health Organization defines menopause as permanent cessation of menstruation due to the loss of ovarian follicular function or surgical removal of ovaries. The abrupt disruption of estrogen is a risk factor for cardiovascular diseases among postmenopausal women. Endothelial dysfunction is an important predictor for determining early atherosclerotic risks.

Aims and Objectives: This study aimed to compare endothelial dysfunction measured by brachial artery flow mediated dilation (BAFMD) in non-obese, non-diabetic post menopausal women with their age-matched menstruating controls.

Materials and Methods: This is a cross-sectional study of 100 women in the age group of 45-55 years attending gynaecology clinic in the department of Obstetrics and Gynaecology in PGIMER Chandigarh a tertiary centre of north India. Parameters recorded were height, weight, BMI, Blood pressure. Biochemical parameters studied were fasting blood sugar, post-prandial blood sugar, lipid profile. Measurement of Brachial artery flow mediated dilation (BAFMD) was done in all patients. Framingham risk score (FRS) was also calculated in all.

Results: The mean age in both case and control was 49.40 ± 3.6 years. Mean BAFMD was 8.334 ± 2.47% in postmenopausal women whereas it was 7.335 ± 3.01% in premenopausal women. The difference was not found to be significant.

Conclusion: The correlation of duration of menopause with FMD made us conclude that endothelial dysfunction increases as the duration of menopause increases.

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1. Introduction

World Health Organization defines menopause as a permanent cessation of menses as a result of loss of ovarian follicular function or surgical removal of ovaries. The median age of menopause is 50–52.76 years. Increased incidence of coronary heart disease is observed in postmenopausal women compared to premenopausal women in the same age range. Endothelial dysfunction is a predictor of early atherosclerotic risk and it can be assessed by the measurement of flow mediated dilation in the brachial artery with Doppler ultrasound. It has been seen that FMD < 4.75% is a risk factor for coronary events. After surgical menopause the risk of coronary heart disease is lower in women taking exogenous estrogens than those who didn’t. Menopause at early age is also associated with increased mortality due to ischemic heart disease. Risk factors for coronary heart disease are significantly higher in postmenopausal women. Endothelial dysfunction is shown to be present in diabetes and obesity. Due to these factors affecting endothelial function our study was aimed at assessing the endothelial dysfunction by brachial artery flow mediated dilation.
flow mediated dilation (BAFMD) in postmenopausal and premenopausal non-diabetic, non-obese females of similar age group coming under low risk by Framingham risk scoring system.

2. Materials and Methods

This study was conducted in the Department of Obstetrics and Gynaecology in the Apex Institute of North India. A total of 100 women in the age group of 45-55 years were recruited for the study after assessing their eligibility according to the selection criteria. A women on hormone replacement therapy, known diabetic and women having any malignancy were excluded. Also women with history of cardiovascular disease, thromboembolic disease and chronic liver and kidney disease were excluded from the study. After inclusion criteria were fulfilled, written informed consent was taken from all women prior to recruitment for this study.

All recruited women were subjected to measurement of brachial artery flow mediated dilation. The subjects 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 minutes. The patient did not ingest substances that might affect FMD such as caffeine, high-fat food and vitamin C nor smoke, for at least six hours 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. Initially the diameter of the brachial artery was determined at rest, and blood flow 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 1) 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 in order 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, for 5 minutes. The ischemia cuff was released after five minutes. The maximum blood flow velocity was detected by analysing mid artery pulsed Doppler signal after 15 seconds of cuff release, while the maximum diameter of the brachial artery will be determined 60 seconds after release. The brachial artery was continuously monitored from 30 seconds before to 120 seconds after ischemia cuff release. (Figure 2) Flow-mediated vasodilation was expressed as the change in post-stimulus diameter as a percentage of the baseline diameter.

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\text{FMD} = \frac{\text{Maximum diameter after cuff release} - \text{baseline diameter}}{\text{baseline diameter}} \times 100
\]

2.1. Statistical analysis

After screening, only the subjects who fulfilled the criteria were included in the study. To detect a statistical significant difference of approximately 4-8% between cases and controls, 50 women were recruited in both groups with a power of 90% and confidence interval of 95%. Quantitative data were presented as mean ± SD or median and interquartile range, as appropriate. For low risk group, mean ± SD or median and interquartile range of FMD was calculated. Normality of data was checked by measures of Kolmogorov Smirnov tests of normality. For normally distributed data means were compared using unpaired t-test and if it was skewed Mann-Whitney test was applied. All calculations were two sided & were performed using SPSS version 15 (Statistical Packages for the Social
Sciences, Chicago, IL). A P value of < 0.05 was considered to indicate statistical significance.

2.2. Approval by the Research and Ethics Committee

The protocol was approved by institute’s Ethical Committee and guidelines set up by ICMR (1994), and Helsinki Declaration (modified 2000) was adhered in all patients enrolled in the study.

3. Results

In this prospective study of 100 women, mean age was 49.40 ± 3.648 year s in both case and control. Table 1 shows subjects in both the groups divided according to their Framingham’s risk score in three groups. Table 2 shows the effect of menopause on various parameters studied in the two groups. Although mean FRS was higher in post-menopausal women, the difference was not found to be significant in our study (p=.143). The mean total cholesterol levels were found to be higher in postmenopausal women and the difference was found to be statistically significant (p=0.001).

The study showed that the mean HDL cholesterol level was higher in post-menopausal women, however the difference was not statistically significant (p=.480). A negative correlation of HDL Cholesterol with duration of menopause (-0.100) was found in our study.

The mean systolic BP in both the groups were found to be almost same. The effect of menopause on endothelial function was studied with the help of BAFMD. The mean BAFMD was higher in postmenopausal women but the difference was not found to be significant in our study (p=.074).

Table 3 The effect of FRS, age, duration of menopause, total cholesterol, HDL, systolic BP on endothelial function was studied. Pearson’s correlation coefficient was calculated.

A negative correlation was seen with FRS and age which was statistically significant and the strength of correlation was moderate. With duration of menopause also negative correlation was seen which was statistically significant but strength of correlation was weak.

Negative correlation of FMD was found with Total Cholesterol (TC) and Systolic BP. Positive correlation was found with HDL cholesterol

4. Discussion

The present study compared the endothelial dysfunction in non-diabetic, non-obese, postmenopausal women, at low risk for cardiovascular disease by FRS score of age group 45-55 years, measured by brachial artery flow mediated dilation (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 as compared to premenopausal women. We did not find any statistical significant difference in endothelial dysfunction measured by BAFMD in both the groups. This made us to conclude that in age matched women with low risk FRS menopausal status does not cause endothelial dysfunction. This was in agreement to 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. The results of the present study are in contrast to the large Framingham’s study in which there was a significant rise in coronary heart disease incidence after menopause. But the major difference between Framingham’s study and our study was that it used 5-year age intervals whereas in our study all the women were age-matched. Smoking was also not included in the analysis, which may have affected the results. Although many other studies conducted in past had shown a significant difference in risks of cardiovascular events in post-menopausal and pre-menopausal women, none of them had taken all age-matched low risk women. So, the non-significant effect of menopause on endothelial function in our patients was most likely due to all of them being in low-risk and age-matched.

Relation of menopause with FRS and different components of FRS was studied. A 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 C Ardiovascular disease) study carried out by N. Agrinier et al in 2009 in which serum total cholesterol and the Framingham 10-year risk of CHD were higher in the post-menopausal 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, blood pressure, fasting glycaemia, triglyceride, serum HDL cholesterol and apolipoprotein A1 levels did not differ according to menopausal status after adjustment for age.

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 was in agreement to the study carried out by Rossi Ret 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. Number of women having cardiovascular risk
Table 1: FRS distribution

<table>
<thead>
<tr>
<th>FRS score</th>
<th>Post-menopausal N (%)</th>
<th>Menstruating N (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-3</td>
<td>5 (10)</td>
<td>4 (8)</td>
<td>9</td>
</tr>
<tr>
<td>4-6</td>
<td>14 (28)</td>
<td>11 (22)</td>
<td>25</td>
</tr>
<tr>
<td>7-9</td>
<td>31 (62)</td>
<td>35 (70)</td>
<td>66</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>50</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 2: Effect of menopause

<table>
<thead>
<tr>
<th></th>
<th>Post-menopausal</th>
<th>Pre-menopausal</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean BAFMD</td>
<td>8.33±2.47</td>
<td>7.33±3.01</td>
<td>p=0.074 (Non-significant)</td>
</tr>
<tr>
<td>Mean FRS</td>
<td>7.22±1.877</td>
<td>6.63±2.038</td>
<td>p=0.143 (Non-significant)</td>
</tr>
<tr>
<td>Mean Total cholesterol</td>
<td>169.94±14.578</td>
<td>159.70±13.080</td>
<td>p=0.001 (Significant)</td>
</tr>
<tr>
<td>Mean HDL cholesterol</td>
<td>47.50±5.534</td>
<td>46.67±6.05</td>
<td>p=0.480 (Non-significant)</td>
</tr>
<tr>
<td>Mean Systolic BP</td>
<td>121.5±9.845</td>
<td>121.2±8.411</td>
<td>No difference</td>
</tr>
</tbody>
</table>

Table 3: Factors affecting endothelial function

<table>
<thead>
<tr>
<th></th>
<th>Correlation with FMD</th>
<th>Coefficient of correlation</th>
<th>Strength of correlation</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FRS</td>
<td>Negative</td>
<td>-0.492</td>
<td>Moderate</td>
<td>p=0.00(S)</td>
</tr>
<tr>
<td>Age</td>
<td>Negative</td>
<td>-0.321</td>
<td>Weak</td>
<td>p=0.00(S)</td>
</tr>
<tr>
<td>Duration of menopause</td>
<td>Negative</td>
<td>-0.281</td>
<td>Weak</td>
<td>p=0.049(S)</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>Positive</td>
<td>0.098</td>
<td>Zero</td>
<td>p=0.568(Ns)</td>
</tr>
<tr>
<td>HDL Cholesterol</td>
<td>Negative</td>
<td>-0.075</td>
<td>Zero</td>
<td>p=0.333(Ns)</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>Negative</td>
<td>0.008</td>
<td>Zero</td>
<td>p=0.460(Ns)</td>
</tr>
<tr>
<td>Smoking</td>
<td>Could not be studied</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

significantly varied according to tertiles of FMD. In the study carried out by Tomiyama H et al they also found that FMD was higher in subjects in the 1st tertile of the FRS range than in patients in either the 2nd or 3rd tertiles of the FRS range. 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 R et al in which age, gender and smoking were independently associated with FMD, but other CVD risk factors were not. However our study was partly in contrast to the study carried out by Konrad T et al, in which they found a significant correlation of HDL cholesterol (p < 0.01) with endothelial function in pre-menopausal women, but in this study they also found a significant correlation of only age and endothelial function in post-menopausal women. 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, and SWAN studies. Direct effect of menopause on FMD has also been studied by Vitale C et al, in which they found that FMD was inversely associated to time from menopause (r=-0.67, P<0.001) and age (r=-0.43, P<0.05).

5. Conclusion

On comparing endothelial dysfunction measured by brachial artery flow mediated dilation (BAFMD) in non-obese, non-diabetic post-menopausal women with their age-matched menstruating controls, in low risk women, menopause did not affect endothelial function significantly. FMD correlated with the cardiovascular risk estimated by FRS and it was affected significantly by age and duration of menopause.

FMD had significant correlation with FRS which is a validated tool for cardiovascular risk assessment. Thus menopausal status does not affect endothelial function in women who are at low risk of cardiovascular disease. The correlation of duration of menopause with FMD made us to conclude that endothelial dysfunction increased as the duration of menopause increases.

6. Financial support and sponsorship

Nil.

7. Conflicts of interest

There are no conflicts of interest.
References


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