

Comparison of asymmetric dimethylarginine levels between pre- and post-menopausal women — a cross-sectional study from Rawalpindi

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Abstract

Objective: To compare serum levels of asymmetric dimethylarginine between premenopausal and postmenopausal women, and to evaluate its relationship with time since onset of menopause.

Methods: The cross-sectional comparative study was conducted at Islamic International Medical College, Rawalpindi, Pakistan, from April 2017 to March 2018, and comprised individuals recruited from the Gynaecology and Obstetrics outpatient department of the Pakistan Railway Hospital in the city. The subjects were divided into two groups; pre-menopausal women in Group I and postmenopausal in Group II. Group II was subdivided into three categories according to time since onset of menopause. Serum asymmetric dimethylarginine levels were estimated in each subject. Data was analysed using SPSS 21.

Results: Of the 80 subjects, there were 40(50%) in Group I with a mean age of 36.25±6.8 years, 40(50%) in Group II with a mean age of 49.83±4.35 years. There was a significant increase in mean asymmetric dimethylarginine levels in Group II compared to Group I ($p < 0.05$). There was also a significant increase in asymmetric dimethylarginine levels with increase in duration since the onset of menopause ($p < 0.001$).

Conclusion: Levels of asymmetric dimethylarginine increased significantly after menopause.

Keywords: Asymmetric dimethylarginine, Premenopausal, Postmenopausal. (JPMA 69: 1808; 2019)

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Introduction

Menopause is a physiological process in every women's life and is defined as absence of menstruation for 12 consecutive months due to loss of ovarian function.¹ Menopause has been proposed as the first step in the causal pathway as hormonal changes eventually result in organ dysfunction.² The incidence of atherosclerotic cardiovascular disease (CVD) in women increases dramatically after menopause.³ Endothelial dysfunction (ED) represents an early reversible stage of atherosclerosis.⁴ Provision of timely and adequate management of CVD calls for early identification of ED with the help of diagnostic tools that are sensitive, non-invasive and easy to access. This is a crucial step for the prevention of atherosclerotic CVD since early detection of ED could be an initial reversible step in the development of atherosclerosis. Making these methods available in clinical practice can provide screening for CVD in the postmenopausal women group.

Asymmetric dimethylarginine (ADMA) is considered an indicator of endothelial dysfunction.⁵ ADMA is an endogenous inhibitor of nitric oxide (NO) synthase (NOS) produced by methylation of specific arginine residues of certain cellular proteins and released when hydrolysis of

these proteins occurs.⁶ It is eliminated from the body by renal excretion (20%) and also metabolised (80%) by hydrolytic degradation to citrulline and dimethylamine (DMA) by the enzymatic action of dimethylarginine dimethyl aminohydrolase (DDAH).^{6,7} Increased concentration of ADMA will compete with L-arginine to be transported into the endothelial cells leading to decreased synthesis of NO and endothelial dysfunction.⁸ In humans, elevated plasma levels of ADMA have been associated with several risk factors related to atherosclerosis, such as hypercholesterolaemia, hypertension (HTN) and aging.⁹ Raised ADMA levels have also been shown to antagonise the endothelium-dependent vasodilation,¹⁰ and recently ADMA was shown to independently predict the levels of intima media thickening in individuals at a varying risk for atherosclerosis.¹¹ ADMA has therefore been discussed as a novel marker for ED and atherosclerosis in humans.⁹ The current study was planned to evaluate the plasma levels of ADMA in postmenopausal women and to compare them to those of premenopausal women.

Subjects and Methods

The cross-sectional comparative study was conducted at Islamic International Medical College, Rawalpindi, Pakistan, from April 2017 to March 2018, and comprised individuals recruited from the Gynaecology and Obstetrics outpatient department of the Pakistan Railway Hospital in the city. After approval from institutional ethics review committee,

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the sample size was calculated using G-power software^{12,13} setting alpha value 0.05, power 0.8 and effect size 0.5 for a two-tailed independent sample t-test. The sample was raised using convenient non-probability sampling technique, and the subjects were divided into two groups; premenopausal women in Group I and postmenopausal in Group II. Group I women included were aged 20-45 years; premenopausal as defined by the occurrence of two menses in the 3 months preceding sampling; no cyclic irregularity in the 12 months preceding testing; non-smoking; weight stability (± 2 kg) during the 6 months prior to testing; and body mass index (BMI) ≤ 30 kg/m². Premenopausal women were excluded if they were pregnant; had a history or current diagnosis of diabetes mellitus (DM), heart disease, HTN, endocrinological abnormalities or other chronic disease; and if they were taking hormone replacement therapy (HRT) or birth control pills. Group II women included were postmenopausal defined as cessation of menstruation for at least 12 months prior to sampling; not more than 4-year postmenopausal; and BMI < 30 kg/m². Time since menopause was defined as the time (in months or years) since menopause established (12 months after the final menstrual period [FMP]). Those exclusion criterion for Group II was identical to Group I. The latter group was subdivided into three categories according to the time since the onset of menopause: those who had experienced menopause for less than two years; those with 2-3 years since menopause; and those with 4 years since menopause. Informed written or verbal consent was obtained from all the subjects. After overnight fasting of 12 hours, 5ml of venous blood was collected from each participant using aseptic technique. The blood was transferred into serum separator tubes (SSTs) and allowed to clot for 30 minutes at room temperature. The blood samples were centrifuged for 10 minutes at 2200 revolution per minute (RPM) for serum separation. The separated serum was transferred to Eppendorf tubes for storage at -70°C till the estimation of ADMA that was determined using commercially available enzyme-linked immunosorbent assay (ELISA) kit (Elabscience Biotechnology).

Data was analysed using SPSS 21. Descriptive data was presented as mean \pm standard deviation (SD). Categorical variables were presented as frequencies and percentages. Independent t test was used to compare ADMA levels between the two groups. Analysis of variance (ANOVA) was used to evaluate the effect of time since the onset of menopause on ADMA levels. $P < 0.05$ was considered statistically significant.

Results

Of the 80 subjects, there were 40(50%) in Group I with a mean age of 36.25 ± 6.8 years, 40(50%) in Group II with a

Table-1: Demographic characteristics.

	Group I		Group II	
	Premenopausal (n=40)		Postmenopausal (n=40)	
	Frequency	Percentage	Frequency	Percentage
Age (Years)				
45 and below	38	95	8	20
46 and above	2	5	32	80
Age at menarche				
11 and below	9	22.5	13	32.5
11.1-12	14	35	12	30
12.1 and above	17	42.5	15	37.5
Body Mass Index				
< 18.5	-	-	-	-
18.5-24.9	32	80	19	47.5
25-29.9	4	10	20	50
30	4	10	1	2.5

Table-2: Mean levels of asymmetric dimethylarginine (ADMA) in pre- and postmenopausal women.

	Group I	Group II	P value
	Premenopausal (n=40)	Postmenopausal (n=40)	
	Mean \pm SD	Mean \pm SD	
ADMA (ng/ml)	443.67 \pm 99.28	557.72 \pm 217.9	0.004

SD: Standard Deviation.

mean age of 49.83 ± 4.35 years. In Group I, 38(95%) women were aged 45 years or below and 2(5%) were aged > 45 years. In Group II, 8(20%) women were aged 45 years or below, and 32(80%) were aged > 45 years.

In Group I, 9(22.5%) women had menarche at an age of 11 years or below, 14(35%) between age 11.1 and 12 years, and 17(42.5%) had menarche after age 12. In Group II, the corresponding numbers were 13(32.5%), 12(30%) and 15(37.5%).

In Group I, 32(80%) women had BMI in the healthy range (18.5-24.9 kg/m²), 4(10%) in the range 25-29.9 kg/m² and 4(10%) had BMI equal to 30 kg/m². In Group II the corresponding values were 19(47.5%), 20(50%) and

Table-3: Association of asymmetric dimethylarginine (ADMA) with duration of menopause.

Time Since Onset of Menopause (Years)	ADMA levels(ng/ml) in postmenopausal group (Mean \pm SD) (n=40)	p value
Less than 2 years	458.6 \pm 177.5	0.001
2-3 years	527.9 \pm 221.7	
4 years	768.1 \pm 114.2	

SD: Standard Deviation.

1(2.5%) (Table-1).

Mean ADMA levels were 443.67 ± 99.28 ng/ml in Group I and 557.72 ± 217.91 ng/ml in Group II ($p < 0.05$) (Table-2).

There was an increase in ADMA levels with increase in duration since menopause ($p < 0.05$) (Table-3).

Discussion

Compared to women in the Western world, women in Asia are subjected to an earlier menopause^{14,15} and all its health-related consequences at an earlier age. After menopause, there occurs a sharp increase in incidence of ischaemic heart disease (IHD) in women^{14,16} which can be ascribed mainly to oestrogen-deficiency and is related pathologies in NO pathway.¹⁴ These changes, along with other contributing factors, result in vascular ED which represents an early reversible stage for developing CVD.¹⁰ This signifies the need for evaluating CVD risk during this early stage of the disease and introducing relevant preventive and treatment modalities so that the burden of CVD on society can be averted. Recently, ADMA has emerged as a novel ED marker. ADMA is an endogenous inhibitor of NOS, and has been considered an independent risk factor for CVD.¹⁷

The current study found that ADMA levels were significantly elevated in the postmenopausal group compared to the premenopausal group ($p < 0.005$). This finding is in accordance with a study¹⁸ that demonstrated an increase in ADMA after menopause due to decreased endogenous oestrogens and subsequent decreased expression of DDAH, an enzyme that catalyses the metabolism of ADMA. Other studies¹⁹ also documented that women show an increase in ADMA levels with the onset of menopause. Similarly, a study²⁰ demonstrated that the health-associated reference interval for measuring ADMA was higher in postmenopausal women compared to the premenopausal. In the current study, the higher level of ADMA in otherwise healthy postmenopausal women can be explained by the emerging role of non-traditional CVD risk factors in women.²¹ The study supports the evidence that CVD risk is increased in postmenopausal women even in the absence of traditional CVD risk factors, like HTN, DM, obesity, smoking, thus highlighting the need to recognise, discuss and treat these emerging risk factors which include elevated ADMA levels.

The possible mechanism of increased asymmetric dimethylarginine levels after menopause is the depletion of endogenous oestrogen stores which results in decreased production of vascular NO. In addition, with falling oestrogen levels, there occurs decreased

expression and decreased activity of DDAH enzyme.²² This is supported by the fact that HRT lowers plasma ADMA levels.²³ However, in clinical practice the use of HRT is recommended only for treatment of vasomotor symptoms in the early menopause period.²⁴

The results of one study²⁵ were contrary to our findings, showing that there was no significant difference in plasma ADMA levels between pre and postmenopausal women. This was attributed to the fact that DDAH hydrolysed more ADMA than NG-mono-methyl-L-arginine (L-NMMA), thus, explaining the higher L-NMMA levels and not ADMA levels in postmenopausal women.

The current study examined the association between ED, as demonstrated by increased serum ADMA levels, and time since onset of menopause. It found a gradual increase in serum ADMA levels with time since onset of menopause in the postmenopausal group. This finding is consistent with a study²⁶ which demonstrated that there was a gradual decline in endothelial-dependent vasodilation, as measured by brachial artery flow-mediated dilation (FMD), across the stages of menopausal transition. Since ADMA is an indicator of ED, a progressive increase in ADMA levels with time since onset of menopause in the current study suggests that a substantial increase in CVD risk occurs as women experience menopause and this risk progressively increases with the duration of the menopause. Since in our study, all the postmenopausal women included were in the early postmenopausal period, according to Stages of Reproductive Aging Workshop (STRAW) criteria,²⁷ our findings of increased ADMA levels in these women suggest that vascular ED starts early in postmenopausal period, indicating a need for screening and prevention therapies to start earlier after menopause.

Conclusion

Serum ADMA levels were significantly increased after menopause which is an ED indicator that represents an early reversible stage of atherosclerotic CVD. Detecting ED at an early stage can be useful in identifying CVD risk in asymptomatic individuals, including healthy postmenopausal women.

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Conflict of Interest: None.

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