

Effect of Hormone Replacement Therapy on Internal Carotid Artery Blood Flow Coefficient and Vaginal IgA

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OBJECTIVE - To assess therapeutic response of hormone replacement therapy (HRT) clinically and by parameters like vaginal IgA and Pulsatility Index (PI) of internal carotid artery in 30 postmenopausal women with natural menopause. **METHODS** - Continuous sequential regime of HRT was given by transdermal estrogen and oral dydrogesterone to 30 study subjects for 3 months. Thirty healthy young women served as controls. Vaginal IgA was measured by paper disk modification of radiodiffusion technique and PI of internal carotid artery was measured by doppler velocimetry. The results were analysed by paired t test in study group before and after therapy and by unpaired t test in study group after therapy and controls. **RESULTS** - There was marked improvement in clinical symptoms. A significant drop in PI from mean value of 2.72 to 1.4 (p value 0.0) was observed, which was comparable to the value observed in controls. Vaginal IgA levels also decreased from mean value of 89.17 to 45.13 (p value 0.0) after the therapy which was slightly higher than the value observed in controls. **CONCLUSION** - PI of internal carotid artery and vaginal IgA are very sensitive, objective and non-invasive markers for assessment of therapeutic response in women on HRT.

Key words: menopause, transdermal estrogen therapy, vaginal IgA, pulsatility index of internal carotid artery

Introduction

Menopause is now recognized as an estrogen deficient endocrinopathy, which results in troublesome neuroendocrine and genitourinary symptoms and long term deleterious effects on musculoskeletal and cardiovascular system. Emerging facts are setting new trends that these effects are potentially curable by hormone replacement therapy (HRT) and the process of accelerated aging can be arrested to some extent¹. There is paucity of data regarding the objective evaluation of response in patients on HRT. A continuous search is being made to find out sensitive, quick and noninvasive markers for proper monitoring and evaluation of therapeutic response to HRT. The present study determines flow coefficient of internal carotid artery by doppler sonography and evaluates the secretory immune response of vagina in postmenopausal women on transdermal estrogen and oral progestogen therapy.

Material and Methods

This prospective study was done over a period of one

year. Thirty postmenopausal women having attained natural menopause and free of any medical and surgical disease were studied. Thirty healthy non-pregnant, parous women aged between 25-35 years served as control. After a preliminary history, examination and base line investigation (hemoglobin, urinalysis, blood sugar, lipid profile), the women in the study group were treated with continuous sequential regime of hormone replacement therapy. Estrogen was given as transdermal therapeutic system (Estraderm TTS - 50 containing 4 mg of 17 β estradiol releasing 50 μ g/day and 21 μ g/cm²/hr of estrogen) twice a week for a period of 12 weeks. The patch was applied over shaved skin of the lower limb in rotatory manner after drying it in air for 15 seconds. Medroxyprogesterone acetate 10 mg orally was added from the 1st to the 12th day of each calendar month. Blood flow coefficient Pulsatility Index (PI) of internal carotid artery was measured in all the study and controls groups by Phillips P-700 machine with 7.5 MHz duplex linear transducer in noiseless surroundings in resting supine position with minimal temperature variations and the women fasting for at least six hours. Gate range used was 6-12 mm and doppler angle was within 30-60^o and doppler power was kept at 0.65 watt. Presence of any stenosis, plaque calcification or luminal irregularity was considered as exclusion criteria. Doppler interrogation of internal carotid artery was done throughout its length.

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PI was calculated by

$$PI = \frac{\text{Maximum velocity} - \text{minimum velocity}}{\text{Time averaged maximum velocity over a cardiac systole}}$$

Vaginal secretion samples from posterior fornix were layered over round discs of 5 mm from an absorbent filter paper. These were stored at -70°C . IgA was determined by paper disc modification of the radial immunodiffusion technique. To 5 ml of 1% agarose gel, 80 μL Dakopatts rabbit antihuman IgA (code No. 092) was added and 6 ml of gel mixture was poured on a glass slide. Paper discs were dipped in the following dilutions of Dakopatts human serum protein calibrator (Code No. 908): 1:20, 1:40, 1:80, 1:160, 1:320 and 1:640. Filter paper discs containing standard (human protein calibrator) and vaginal secretions were placed on the surface of gel slides containing antihuman IgA and were incubated at 37°C for 72 hours. After repeated washing and pressing, the slides were dried and stained with Coomassie brilliant blue solution, followed by destaining. The diameter of precipitation rings was measured accurately to 0.1 mm using calibrated magnifier. The squared diameters of standard precipitation rings were plotted against the amount of IgA and a calibration curve was obtained. By interpolation of curve vaginal IgA levels were measured. The statistical analysis was done using paired t test in the study group before and after therapy and unpaired t test in study group after HRT and control group.

Results

The mean age of menopause and mean age of study group was 46.3 and 49.7 years respectively. The mean body mass index was 22.2 kg/m^2 . The commonest symptoms noted were anxiety (77%) and irritability (77%). The improvement in these symptoms ranged from 73%-100% (Table I). Loss of libido (60%) and dyspareunia (50%) were the most frequently encountered genitourinary symptoms. The improvement in these ranged from 84%-100% (Table II).

Table-I: Effect of HRT on Neuroendocrine Symptoms

Symptoms	Baseline	After 12 wks
Hot flashes	50%	6.7%
Night sweats	50%	3.3%
Insomnia	50%	3.3%
Mood changes	43%	0
Anxiety	77%	6.7%
Irritability	77%	6.7%
Loss of memory/concentration	73%	20%
Depression	57%	6.7%

Table - II: Effect of HRT on Genitourinary and Miscellaneous Symptoms

Symptoms	Baseline	After 12 weeks
Genitourinary		
Dyspareunia	50%	3.3%
Loss of libido	60%	10%
SUI	20%	3.3%
Vaginal infections	30%	0
UTI	33%	0
Urge incontinence	10%	0
Vaginal dryness	10%	0
Miscellaneous		
Joint aches	67%	13.3%
Paraesthesias	60%	6.7%
Dry skin/hair/nails	23%	0

The PI values of left and right internal carotid artery (ICA) were correlated in each woman and mean PI of left and right side was considered for analysis. There was no significant difference in PI of the left and right sides (P value 0.771). After therapy for 3 months, PI values of left and right ICA were again comparable. After 12 weeks of transdermal estrogen therapy there was a reduction of mean PI by 49% (p value =0.0).

The mean PI value after therapy was comparable to the PI of control (p value=0.0). This implies a marked reduction in resistance to blood flow and therefore an improvement in blood flow across internal carotid artery (Table III).

Table - III: Pulsatility Index of Internal Carotid Artery

Pulsatility Index	Study group Before	After	P value	Control	P value
Left ICA	2.73 \pm 0.39	1.50 \pm 0.42	0.0	1.31 \pm 0.22	0.087
Right ICA	2.70 \pm 0.46	1.30 \pm 0.31	0.0	1.39 \pm 0.18	0.087

^a Comparison with study group after therapy

Vaginal IgA levels were evaluated in 24 women as 6 women were excluded from the study due to poor paper disc formation. The IgA levels reduced by 49% in the study group (Table II). This reduction was statistically significant (p value=0.0). However the vaginal IgA level after treatment in the study group was still higher than that observed in controls (Table IV).

Table IV : Vaginal IgA

Vaginal IgA	Study group		P value	Control group
	Before	After		
Mean levels	89.17±24.37	45.13±22.60	0.0	24.80±8.4

Discussion

In the present study, the mean age of menopause was less than what is observed in other studies. Continuous sequential regime of HRT was chosen because the study subjects were mostly less than 55 years of age and they did not object to withdrawal bleeding. The acceptability rate was 100% after proper counseling.

Assessment of cardiovascular function due to estrogen deficiency has been a subject of interest, which poses various difficulties, as there are very few studies on objective assessment during continuation phase of therapy. Available epidemiological data has been on surveying cardiovascular risk factors (like hypertension, smoking obesity and diabetes mellitus), lipid profile² and coronary angiography. The epidemiologic surveys estimate that not more than 20-25% cardioprotection is offered by change in lipid profile³ and that angiography being an invasive procedure cannot be repeated frequently⁴. Doppler ultrasound is a noninvasive marker for evaluation of blood flow. Many indices of waveform analysis have been devised but only pulsatility index (PI) and resistance index (RI) are in clinical use. RI may be increased by vascular stenosis or any disease in the organ supplied by the vessel. Therefore PI was used as a blood flow coefficient in this study. Exogenous and endogenous estrogen is reported to increase the blood flow in coronary and extracoronary arteries. Internal carotid artery being easily accessible was chosen for determining PI. In the present study, after 12 weeks of HRT, mean PI decreased remarkably and the values almost paralleled to those of controls. Penotti et al⁵ reported reduction in PI of ICA and middle cerebral artery but to lesser extent. Reduction in PI has also been studied in the uterine artery⁶.

Genitourinary system is most sensitive to alterations in estrogen levels. Decrease in estrogen induces bacterial colonization in the vagina, which in turn produces secretory immune response (IgA) as a local defense mechanism against infection^{7,8}. In the present study, vaginal IgA decreased by almost 50% from a mean value of 89.17 to 45.13. Its value in control group was still lower than post-therapy values. Since the study period was 3 months, it could be possible that

further reduction in vaginal IgA could result if the study period was prolonged. Milson et al⁸ treated postmenopausal women with local estriol cream and observed that mean value of vaginal IgA was (41.5 mg/L) which was higher than the mean value (16.4 ml/L) in fertile women.

No major untoward effects of transdermal estrogen were noted in our study. Local rash was observed in 4 out of 30 women. Progestogen withdrawal bleeding occurred in 25 out of 30 women, which was an accepted effect. Breast tenderness, fluid retention and facial swelling were seen in two patients during the progestational phase. No woman complained of breakthrough bleeding or gastrointestinal disturbances.

References

1. Barlow DH. Hormone replacement therapy and other menopause associated conditions. In: Khaw KT. *British Medical Bulletin: British Council, Churchill Livingstone 1991; 48:356-67.*
2. Walsh BW, Shiff J, Rosner B et al. Effects of postmenopausal estrogen replacement therapy on the concentrations and metabolism of plasma lipoproteins. *N Engl J Med 1991; 325: 1196-1204.*
3. Bush TL, Connor BE, Cowan DK. Cardiovascular mortality and noncontraceptive use of estrogen in women. Results from lipid research clinic followup study. *Circulation 1987; 75:1102-9.*
4. Brown PM, Johnston KW. The difficulty of quantifying the severity of carotid stenosis. *Surg 1982; 92: 468-73.*
5. Penotti M, Nencioni T, Gabrielli L et al. Blood flow variations in internal carotid and middle cerebral arteries induced by postmenopausal hormone replacement therapy. *Am J Obstet Gynaecol 1993; 169: 1226-32.*
6. Hillard TC, Boume TH, Whitehead MI et al. Differential effects of transdermal estradiol and sequential progestogen on impedance to blood flow within the uterine arteries of postmenopausal women. *Fertil Steril 1992; 58:959-63.*
7. Wira CR, Stern JE. Estradiol regulation of the secretory immune system in the female reproductive tract. IgA in uterine and vaginal secretions of rats following portacaval anastomosis. *J Steroid Bio Chem 1986; 24: 33-7.*
8. Milson I, Nilson LA, Brandberg A et al. Vaginal immunoglobulin A levels in postmenopausal women: Influence of oestriol therapy. *Maturitas 1991; 13: 129-35.*