# EFFECT OF FOLIC ACID SUPPLEMENTATION ON HOMOCYSTEINE LEVEL IN POSTMENOPAUSAL WOMEN

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**BACKGROUND:** Hyperhomocysteinemia is an independent risk factor for atherosclerotic diseases including Ischaemic heart disease, stroke and peripheral vascular disease. Homocysteine (Hcy) is an intermediate formed during the catabolism of essential sulphur containing amino acid methionine, increased Hcy is associated with endothelial dysfunctions in healthy human. Plasma Hcy is significantly lower in premenopausal women than young men but after menopause basal homocysteinemia increases significantly in women approaching those in men. Several studies showed that hyperhomocysteinemia to be stronger risk factor for CHD (Coronary Heart Disease) in women than men. It seems likely that altered hormonal status and age related low folate intake are responsible for this. The present study was designed to evaluate the effect of folic acid supplements for six months, on Hcy level in postmenopausal women. **Methods:** Hcy was estimated by Flourescence Polarization Immunoassay (FPIA). **Results:** There was a significant ($p$<0.001) decrease in Hcy level after six months of folic acid supplements. **Conclusion:** Hcy is an independent risk factor for atherosclerotic disease, this study favours the view that after menopause Hcy level increases significantly and a simple non Toxic and relatively inexpensive vitamin (folic acid) intervention might be useful in primary cardiovascular prevention in this high risk group because Hcy is a stronger risk factor for CHD in postmenopausal women than men.

**Keywords:** Menopause, Homocysteine, Folic acid.

## INTRODUCTION

Homocysteine (Hcy) is an intermediate formed during the catabolism of sulfur containing essential amino acid, methionine.[1,2,3] It is found either as free homocysteine, cysteine-homosysteine mixed disulfide or protein bound homocysteine. The one that is bound with protein in plasma reflects total plasma homocysteine (tHcy).[4]

Gender, age and circulating levels of folate and B12 effect plasma tHcy level[5] and also by oestrogen status possibly.[6]

Hcy concentration rises progressively with age in men and woman, likely culprits include clinical or subclinical folate and $B_{12}$ deficiencies.[1,2]

Increased Hcy is associated with endothelial dysfunctions in healthy human.[7-9] Endothelial dysfunctions are manifested by impaired endothelial dependent regulation of vascular tone and blood flow, by increase recruitment and adhesion of circulating inflammatory cells to endothelium and by a loss of endothelial cell antithrombotic function contributes to vascular disorder linked to increase Hcy.[10,11]

Hyperhomocysteinemia is an independent risk factor for atherosclerotic diseases, including ischaemic heart disease, stroke and peripheral vascular disease.[6,10,12-15]

Individuals with elevated level of Hcy tend to have higher incidence of cardiovascular disease.[11,16,17]

Hyperhomocysteinemia is considered to be a stronger risk factor for CHD in woman than men.[18]

Reiss *et al*., 1999[19] found basal homocysteinemia is significantly higher in men than women. After menopause basal homocysteinemia increases significantly in women, approaching those in men.

This is most probably due to oestrogen deficiency because in young woman where oestrogen production is high, serum lipids and Hcy levels are normal.[17,20] But after menopause abnormal lipid profile and hyperhomocysteinemia and increase incidence of CHD show a possible relationship among oestrogen, normal lipid profile, normal tHcy levels and relative immunity to CHD.[20,21]

From all this it becomes evident that premenopausal women are protected from CHD by having favourable lipid profile and plasma tHcy level. After menopause this protection is lost most probably due to oestrogen deficiency. Hormone replacement therapy (HRT) was the focus of medical research for the last 10–15 years, initially it was observed that it protects postmenopausal women from CHD but now it is proved that follow up results are much harmful. It is not justified to expose a patient to so many serious diseases just to protect them from CHD.

Simply folic acid supplements can be beneficial to postmenopausal women in protecting them from CHD. So interference to lower it with medication with least side effects will be a great help to postmenopausal women to whom Hormone Replacement Therapy should no more be prescribed due to its harmful effects.

Considering this we designed the present study to evaluate the level of tHcy in pre- and post-menopausal women, and also looked for the effects of Folic Acid Supplementation (1 mg/day) on tHcy level in postmenopausal women. This type of study was not conducted at least in local population before.

## MATERIAL AND METHODS

The subjects included in the study were randomly selected from women attending different Out Patient Departments (OPDs) of Ayub Teaching Hospital Complex and District Headquarter Hospital, Abbottabad. Informed written consent was obtained on voluntary basis from all the subjects included in the study.

Thirty pre-menopausal and thirty post-menopausal women were included in the study. Both pre- and post-menopausal subjects included in the study were otherwise normal and were not suffering from any disease such as, diabetes mellitus, coronary heart disease, kidney disease etc. Subjects taking multivitamins and in particular folic acid were excluded.

Five ml of venous blood was collected from each pre- and post-menopausal subject after an overnight fast of 12–14 hours. Serum was separated and stored at -20 °C until analyzed for Hcy. Post-menopausal women were given 1 mg Folic Acid per day orally for a period of six months. At the end of folic acid supplementation period 5 ml of blood was again collected after an over night fast, serum was separated and stored until analyzed.

Estimation of total L-homocysteine in human serum or plasma was done by Flourescence Polarization Immunoassay (FPIA) using pre-packed Kits (catalog No. B3D390, 33-0781/R5) on the IMx Analyser.

Mean and Standard Error of the Mean (SEM) were calculated. Results from different group of subjects were compared using student's *t*-test and the level of significance was set at $p<0.01$.

## RESULTS

The results of this study are summarized in Table-1 and 2. The age range of the subjects was 31–33 years for premenopausal women and 54–56 years for postmenopausal women. Serum tHcy level in premenopausal women was observed to be 9.75±0.25 µmol/L as compared to 17.90±0.37 µmol/L in untreated post-menopausal women. tHcy level in premenopausal women is obviously significantly lower ($p<0.001$) than postmenopausal women. Six months subsequent to folic acid supplementation (1 mg\day) in postmenopausal women, serum tHcy level decreased to 14.63±0.35 µmol/L. This decrease although small is statistically significant ($p<0.001$) and may be appreciated in a glance in Figure-1.

**Table-1: Age and Total Homocysteine levels (µmol/L) in Pre- and Post-menopausal women.**

| | Pre-menopausal | Post-menopausal | *p*-Value |
|---|---|---|---|
| **Age** | 32.1±1.14 | 55.47±0.89 | $p<0.001$ |
| **tHcy** | 9.75±0.25 | 17.90±0.37 | $p<0.001$ |

Values are expressed as mean±SEM.

**Table-2: tHcy level (µmol/L) before and after folic acid supplements in postmenopausal women.**

| | Initial | After 6 months | *p*-Value |
|---|---|---|---|
| **tHcy** | 17.90±0.37 | 14.63±0.35 | $p<0.001$ |

Values are expressed as mean±SEM.

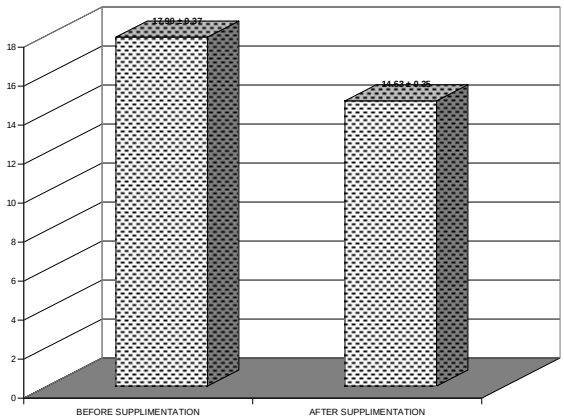


**Figure-1: Before and after supplementation of Folic Acid in Post-menopausal**

## DISCUSSION

Our study showed a highly significant decrease in tHcy level following six months of 1mg/day folic acid supplements in postmenopausal women. A number of studies support this finding.[3,7,14,22,23]

Plasma Hcy is significantly lower in premenopausal women than young men.[19,24]

Blom *et al.*, 1988[24] found significantly higher concentration both in serum and urine of transamination metabolite in premenopausal women than group of men, this higher methionine transamination in premenopausal women may contribute to keep Hcy level low.

Study conducted by wouter *et al.*, 1995[5] concluded that plasma Hcy concentration is significantly higher in post menopausal women as compared to premenopausal women having higher concentration of serum 17 β-oestradiol measured simultaneously. Hak *et al.*, 2000[25] concluded that plasma Hcy is affected by menopause. After menopause high Hcy level seems to be the sum of to altered hormonal status and low folate level (inadequate age related intake).

Because it is proved that folic acid supplementation either individually or in combination

with HRT has beneficial effect in lowering Hcy Level in low oestrogen status subsequent to ovariectomy.[14,26,27]

Moderate hyperhomocysteinemia, defined as total homocysteine concentration between 12 to 13 µmol/L, represents as independent risk factor for heart disease, vascular brain disease, phlebothrombosis and thromboembolic complications. It is related to placental abruptions, spina bifida and some neuropsychiatric disorders. Hyperhomocysteinemia is metabolic syndrome based on interaction between genetic factors diseases and demographic factors (smoking, aging, hormonal and nutritional factors). Moderate hyperhomocysteinemia occurs in about 20 to 30% of patients with clinical complications of atherosclerosis. Prospective and genetic studies have shown, that moderate hyperhomocysteinemia in healthy persons is only a weak predictor of cardiovascular diseases. Contrary to it, in patients with ischaemic heart disease, renal failure or diabetes mellitus and in thromboembolic disease, hyperhomocysteinemia represents a strong predictor of vascular mortality and morbidity.

Treatment of hyperhomocysteinemia is based on the administration of pharmacological doses of folic acid, which can decrease total homocysteine concentration by 25 to 30%. Such decrease, which is in average 3 µmol/L, results in the decrease of relative risk of ischaemic heart disease by 11 to 16%, phlebothrombose by 25% and vascular brain diseases by 19 to 24%.[3,28,29] In recent years great attention has been focused on the role of folates on public health. Folate prevents the development of neural tube defects and reduce risk of coronary heart disease, some kinds of cancer and neuropsychiatric disorders.[12,22]

Several studies that included both men and woman found hyperhomocysteinemia to be a stronger risk factor for CHD in woman than men.[18]

Co administration of vitamin $B_{12}$ may be needed to prevent irreversible neurogical damage.[1]

All Patients with known coronary artery disease should take prescription strength of folic acid(1 mg/day) which has few if any known adverse effects.[30] Study conducted by Neal *et al.*, 2002[31] determined the effect on Hcy level of two doses of folic acid where high dose is typical of that provided by pharmacological intervention and low dose approximates that provided by dietary supplementation. They concluded that high dose pharmacological supplementation produced greater reduction of Hcy level than lower dose ($p$=0.01). Moreover they recommended that high dose pharmacological supplementation would produce greater reduction for high risk individuals such as post menopausal women.[31]

Intervention to lower serum homocysteine, if judged to be worthwhile, should not be limited to people with a high homocysteine but should be offered to everyone at high risk, regardless of pre-treatment homocysteine.

In third world countries like Pakistan where majority of patients attending OPDs of government hospitals belong to low socio economic group and have joint family system dependent upon one male member (earning), and lot of dependent children. It is evident that in such families women are mostly mal nourished. So it is recommended that particularly post menopausal women should be convinced and prescribed folic acid supplementations without prior costly estimation of tHcy level because such supplementations are very cheap, without side effects and are highly effective.

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