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Effect of Folic Acid on Serum Homocysteine Levels in Patients with Cardiovascular Diseases (CVD)

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ABSTRACT

Objective: To evaluate the effect of 5 mg folic acid supplement for five months, on serum homocysteine (Hcy) level in patients with cardiovascular diseases (CVD). **Methods:** A total of 50 patients and 25 control subjects were studied. Out of 50 CVD patients 31 were men and 19 were women. Controls were age and sex matched. Serum Hcy level were measured in both patients and controls at the initiation of study. After treatment with folic acid for five months, serum Hcy level was measured in patients. **Results:** Total serum Hcy level before treatment with folic acid in patients was 22.91 ± 2.94 µmol/L and in controls 14.4 ± 1.39 µmol/L. Total serum Hcy level after consumption of folic acid for five months in patients was 14.63 ± 2.935 ($P<0.001$). There was significant reduction in low density lipoprotein (LDL), total cholesterol (TC) and Blood pressure (BP) in folic acid treated group as compared to those not receiving folic acid. **Conclusion:** This study recommends screening for homocysteine level in normal individuals for early detection of cardiovascular diseases (CVD) and folic acid supplementation in CVD patients with Hyperhomocysteinemia (HHC) to avoid cardiovascular complications.

INTRODUCTION

Cardiovascular disease includes Coronary heart disease (CHD) and Stroke [1]. Cardiovascular disease (CVD) is responsible for about 30% of all deaths worldwide, with about 80% of total deaths occurring in developing countries [2]. In people with a previous heart attack or stroke, without any treatment, cardiovascular disease mortality is about 5% per year for life. About half of all cardiovascular deaths occur in individuals with previous myocardial infarction or cerebral thrombosis. All such individuals should be offered treatment to reduce the reversible risk factors and would benefit from taking Polypill (statin, three blood pressure lowering drugs at half of their standard doses, folic acid and aspirin) [3]. Recent studies beside demonstrate that diabetes, hypertension, hypercholesterolemia, cigarette smoking and positive family history well known risk factors in the atherosclerosis phenomena, high plasma level of homocysteine also acts as an independent risk factor of atherosclerosis and coronary artery disease [4].

Hyperhomocysteinemia is usually defined as an elevation of plasma total homocysteine >15 µmol/L and may be caused by genetic defects, renal insufficiency, certain drugs, or nutritional deficiencies of folate, vitamin B_6, or vitamin B_{12}. Normal total homocysteine (tHcy) levels range between 5 and 15 µmol/l with elevations of 16 to 30 µmol/l, 31 to 100 µmol/l, and >100 µmol/l classified as mild, moderate, and severe hyperhomocysteinemia respectively [5].

Homocysteine is a sulfur-containing amino acid produced in the metabolism of the essential amino acid methionine, an essential amino acid derived from dietary protein. Homocysteine is metabolized through two vitamin-dependent pathways viz. remethylation (requiring folate and vitamin B_{12}), which converts homocysteine back to methionine, and transulfuration (pathway requiring vitamin B_{12}), which converts homocysteine to cysteine and taurine and a second remethylation pathway in the liver and kidney utilizes betaine instead of folate (folate independent) [5]. The total plasma (or total serum) homocysteine (tHcy) reflects the combined pool of free, bound, reduced, and oxidized forms of homocysteine in the blood.

An increase in homocysteine can be brought about by number of factors involved in this metabolic pathway. Metabolic reasons for this increase are often linked to dietary intake of methionine or other vitamin cofactors [6, 7]. Modest elevation of this amino acid is also seen in persons with deficiency or low intake of vitamins (folate and B_{12} both cofactors in the

metabolic pathway) that leads to decreased activity of the respective enzymes for which these vitamins are cofactors.

The methionine tolerance in some people could be altered due to some other factors such as genetic makeup. Genetic defects in synthesis of certain enzymes in the methionine catabolic or methionine remethylation pathway contribute in a major way. Methylene tetrahydrofolate reductase (MTHFR), enzyme responsible for remethylation of homocysteine to methionine has been cloned and sequenced with disease associated mutations identified. In particular, a C → T substitution at nucleotide 677 results in a conservative Ala → Val replacement accounting for thermo labile MTHFR enzyme (tMTHFR). tMTHFR genotype is significantly correlated with decreased enzyme activity and increased homocysteine levels. The hyperhomocysteinemic effects of tMTHFR are variable and appear to be related to folate sufficiency [8].

Folic acid, a water soluble vitamin B, has recently gained considerable attention because of its great potential to prevent many disorders through supplementation for the general population. Folate (main circulating metabolite of folic acid in plasma is 5 methyl tetrahydrofolate reductase (5MeTHF) acts through at least four mechanisms in atherosclerosis: *(i)* indirectly, to decrease homocysteine level and insure optimal functioning of the methylation cycle, *(ii)* directly, to produce antioxidant effects, *(iii)* to interact with enzyme endothelial nitric oxide synthase (eNOS), and *(iv)* to affect cofactor bioavailability of nitric oxide [9].

Several dietary and lifestyle factors, genetic defects, nutritional deficiencies, and other etiologies can cause elevations in homocysteine (Genetic enzyme polymorphisms MTHFR, methionine synthase, cystathionine β synthase, dietary deficiency, folic acid, vitamin B_{12}, vitamin B_6, methionine, lifestyle factors, chronic alcohol intake, smoking, high coffee intake, renal failure, end-stage diabetes, systemic lupus erythematosus, hyper proliferative disorders, medications [methotrexate, sulfonamides, antacids]). A thermolabile variant of methylene tetrahydrofolate reductase (MTHFR) with reduced enzymatic activity (C677T mutation) is the most common form of genetic hyperhomocysteinemia (5% to 14% of the general population is homozygous for this mutation). However, an association of this mutation with increased CVD risk is manifest only in populations characterized by low baseline folate levels. Deficiency of folic acid, vitamin B_6, and vitamin B_{12} accounts for the majority (two-thirds) of cases of elevated homocysteine in the general population. There is currently insufficient evidence to recommend routine screening and treatment of elevated homocysteine

concentrations with folic acid and other vitamins to prevent atherothrombotic vascular disease [5].

The present study was designed to investigate the effect of folic acid on serum homocysteine levels in patients with cardiovascular diseases and to stress the need for screening for homocysteine as part of the risk assessment profile.

MATERIALS AND METHODS

Study population

The study of effect of folic acid on serum homocysteine levels in patients with cardiovascular diseases was conducted in Vivekananda General Hospital, Tiruchengode and a private hospital, Coimbatore. Cardiovascular disease patients of both gender above 45 years of age (50 patients) who were admitted to the Coronary Care Unit with symptoms of chest pain were enrolled into study. Myocardial infarction (MI) was confirmed using a standard 12–lead ECG. Abnormal 12–lead ECG, absence of vitamins/supplements and prescribed drugs such as anticonvulsants/antiepileptics, no renal disorders (confirmed by urea/creatinine levels) and angiographically proven stenosis (>50%) in two or more vessels are accounted as the inclusion criteria. Age and sex matched healthy individuals (n=25) without clinical evidence of coronary artery disease or family history of cardiac problems and with normal ECG constituted the control group. Inclusion criteria were based on factors such as family history for cardiac problems and normal resting 12 lead ECG. Subjects with renal failure (Creatinine ≥ 3.0gm/dl), hepatic dysfunction, pregnancy, hypothyroidism, and those taking methotrexate, hydrochlorthiazide, carbamazepine or phenytoin were excluded from the study. Institutional Research and Ethics committee approved the study and issued a letter of permission to conduct study. Blood samples were collected after overnight fasting of more than 10 hrs. Plasma and serum samples were separated and preserved in accordance with instructions of manufacturers test reagent kit. Serum homocysteine level was measured at the initiation of study in CVD patients and controls. All the subjects mentioned above gave their consent in writing, and the objectives of the study were fully explained to them in detail prior to taking consent. Thereafter study participants were allocated into two groups such as Patient group and control group. CVD patients were included in patient group and normal age sex matched controls were included in Control group. CVD patients were given 5 mg folic acid per day orally for a period of five months in addition to their drug regimen (Ramipril 5 mg, aspirin 75 mg and atorvastatin10 mg / metoprolol 50 mg, amlodipine 5 mg, aspirin 75 mg and

atorvastatin 10 mg). At the end of five months of folic acid supplementation, serum homocysteine level was measured in CVD patients. Serum total homocysteine was measured using Enzyme linked Immuno sorbent assay as described by Fantzen et al using axis homocysteine EIA Kit from Ranbaxy Diagnostic Ltd., India. Normal serum homocysteine reference values for adult male and females lies between 5 and 15 µmol/L. Standards ranging from 2.0 – 50.0 µmol/L were used to obtain a five-point calibration curve required for the assay of homocysteine. This method not only measures free homocysteine but also homocysteine-homocysteine disulfides, homocysteine-cysteine disulfide and protein bound forms. Another 10 ml of blood sample collected in polypropylene tubes were used to obtain serum for estimation of serum total glyceride (TG), total cholesterol (TC), and high density lipoprotein (HDL) using GOD PAD method by fully automated clinical chemistry analyzer (Hitachi 912, Boehringer Mannheim, Germany). Serum low density lipoprotein cholesterol (LDL-C) was estimated from the primary measurements using the empirical of Friedewald et al. Serum LDL-C = TC- HDL-C-TG/5. Serum glucose, urea, and creatinine were measured using diagnostic kits made by Roche Germany and Hitachi 912 random access chemistry analyzer.

Statistical Analysis

All results were analyzed using computerized statistical package Graphpad Instat software. Values are represented as Mean ± SD (standard deviation). Paired t test and unpaired t test were used to calculate statistical significance. Statistical significance was noted as $p < 0.05$ at 95% confidence interval.

RESULTS

Demographic, behavioral and nutritional variances in patient and control groups are presented in Table 1. The mean age of the patients group (n=50) was 52.9 ± 4.699 and for Control group (n=25) was 49 ± 2.404. The patient group consisted of 62% males and 38% females. In Control group, 60% were males and 40% females. In the patients group 78% were non-vegetarians and 14% were alcoholics, in control group 80 % were non-vegetarians and 12% alcoholics. 72% of patients were presenting with angina, 18% with acute myocardial infarction (AMI), 10% with stroke and all the 50 patients were hypertensive and hyperlipidemic.

Table 2 shows the levels of serum homocysteine, total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL) and low density lipoprotein (LDL), systolic blood pressure (SBP) and diastolic blood pressure (DBP) in patient and control groups. TC, TG, LDL, SBP, DBP and serum homocysteine levels in CVD patients group were statistically different when compared with control group. Mean homocysteine levels were raised (22.91 ± 3.125 µmol/L) in patient group compared to control group (14.41 ± 1.39 µmol/L) ($p<0.0001$). From the results obtained, it is clear that there is a relationship between elevated levels of homocysteine and cardiovascular diseases (CVD).

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%. In recent years great attention has been focused on the role of folates on public health and it is reported that folate prevents the development of neural tube defects and reduces risk of coronary heart disease, some kinds of cancer and neuropsychiatric disorders [10].

According to Stehouwer, fasting hyperhomocysteinemia (HHC) can be treated with folic acid 1-5 mg/day [11, 12]. In published studies, there is no indication on optimal dose of folic acid for decreasing serum homocysteine level but different studies used a range of doses of 0.5 mg/day to 10 mg/day [13, 14]. In our study folic acid 5 mg/day, an intermediate dose was arbitrarily selected. Our study showed a highly significant decrease (mean reduction of 8.35 µmol/L, $P<0.0001$) in tHcy level following five months of 5 mg/day folic acid supplements in patients with CVD and our findings are in accordance with earlier reports [10, 15].

Change in lipid profile, blood pressure on treatment with folic acid in CVD patient group are shown in table 3. The mean serum total cholesterol (TC) was significantly reduced from 217.5 ± 11.83 to 196.48 ± 13.84 mg/dL ($P=0.0008$) in CVD patients after treatment with folic acid for 5 months. Significant reduction in mean LDL was observed in CVD patients after treatment with folic acid ($P=0.004$). Reductions in mean level of TG ($P=0.0709$) and HDL ($P=0.2515$) in CVD patients were insignificant after folic acid therapy for five months. At the end of study, there were significant reductions in both SBP and DBP of CVD patients ($P<0.0001$) taking folic acid.

Table 4 shows the mean homocysteine levels between the different age population in the control and CVD patient groups. The mean serum homocysteine levels of CVD patient population in 45-50, 50-55, 55-60 and 60-65 years age group were 18.69 ± 3.08; 19.4 ± 3.10; 24.52 ± 3.24 and 25.52 ± 2.94 µmol/L respectively. The mean serum homocysteine in the control population in the age group 45-50, 51-55, 55-60 and 60-65 years were 13.01 ± 2.162; 14.21 ± 2.644; 15.27 ± 2.89 and 15.46 ± 2.96 µmol/L respectively. The homocysteine levels in patient group were significantly different (P<0.0001) from the control group in the respective ages. It was observed from the results that there is a marked increase in the mean serum homocysteine level with increasing age in patients when compared with controls (Table 2). These findings are in concordance with Bejoy Baby et al [8] and Nygard et al[16].

The mean homocysteine levels in the patient population based on the behavioral and clinical factors are shown in Table 5. The difference in mean homocysteine level was significant among smokers and non-smokers in patient group (P<0.0001). Both alcoholics and non-alcoholics showed high homocysteine level as compared to control group (P<0.0001). Interestingly smokers and alcoholics in patient group were found to have high homocysteine level when compared with their counterparts signifying influence of both risk factors on homocysteine and CVD.

Mean homocysteine of vegetarian in control group showed slight elevation however not significant when compared with non-vegetarian. Although vegetarian and non-vegetarian CVD patients showed high homocysteine values as compared to control, vegetarians in patient group comparatively had greater levels of homocysteine (24 ± 2.67 Vs 20.83 ± 3.07 µmol/L;P = 0.0032) (Table 5). Our results are indicative of high risk of hyperhomocysteinemia for vegetarians.

Table 6 shows the mean homocysteine levels in the patient population based on treatment with folic acid for 5 months. Significant reduction of serum homocysteine was observed at the end of study in CVD patient group. Mean level of serum homocysteine was significantly reduced from 22.91 ± 3.125 to 14.563 ± 2.19 µmol/L in CVD patients (P<0.0001) (figure). There were significant reductions of serum homocysteine level observed in smokers, non-smokers, alcoholics, non-alcoholics, patients with angina, acute myocardial infarction (AMI) and stroke when compared to same population before treatment with folic acid.

DISCUSSION

In recent years, many of the researcher's attention is focused on serum homocysteine levels and their role on cardiovascular diseases and globally trials are going on in relating the role of homocysteine and whether reduction of homocysteine would help to reduce cardiovascular disease incidence and complications. In this context, we have attempted in our study, to see whether reduction of homocysteine by folic acid supplementation would help to reduce risk factors such as blood pressure and lipid levels in CVD patients. Our study shows significant ($P<0.001$) increase in serum homocysteine levels before treatment with folic acid in CVD patients as compared to controls.

Based on the results of this study and other previous study (Brilakis et al) it seems that hyperhomocysteinemia is a risk factor for premature CAD in men but not proven so in women [17]. There was no significant difference in serum homocysteine between male and female subjects before treatment with folic acid. However previous study reports higher baseline serum Hcy level in men compared to women [13]. The findings of our study showed that there was significant reduction in serum Hcy level in both men and women ($P<0.0001$) after folic acid treatment.

Cigarette smoking is known to be associated with a raised plasma Hcy level [16, 18, 19]. Such a phenomenon was also observed in our patients. (Smokers 24.5 ± 1.98 vs non-smokers 20.32 ± 2.99 µmol/L ($P<0.0001$). Nicotine and carbon monoxide in smoke separately produce tachycardia, hypertension and vasoconstriction and both produce direct endothelial damage. Smoking also affects vaso occlusive factors such as platelet aggregation, plasma viscosity and fibrinogen levels [20]. Smokers also tend to have lower levels of the vitamin B, folate, vitamin B_6 and vitamin B_{12} all of which affect homocysteine levels by acting as co-factors (vitamins B_6 and B_{12}) or co-substrate (folate) for the enzymes controlling homocysteine metabolism [9, 20, 21, 22].

Chronic alcoholism is associated with hyperhomocysteinemia. Our study findings also prove this fact; alcoholics with CVD showed hyperhomocysteinemia. The aetiology of folate deficiency in alcoholism is described to several causes such as low dietary intake, poor absorption, decreased hepatic uptake and retention and increased urinary excretion of folate. Therefore alcoholism may have additive effect in raising homocysteine levels along with role of homocysteine in CVD [23].

Body turns much of homocysteine back into methionine with the help of vitamin B_{12}. If someone is B_{12} deficient, homocysteine levels will increase because this relation cannot take place. On the other hand, there are other studies, which mentioned the "Indian dietary paradox". The assumption is that many Indians by virtue of their vegetarian diet do receive fairly adequate amounts of folate. In that case, the reasons for this paradox is prolonged cooking, heating, frying etc of vegetables (which is a common practice among Indian households) which can destroy up to 90% of the folate content of vegetables [24]. In most non-vegetarians with elevated homocysteine, folate is more of a problem than in vitamin B_{12}. Since vegetarian diets are typically high in folate, elevated homocysteine levels in vegetarians are normally due to a low vitamin B_{12} intake; this fact has been proved in a North Indian study [25, 26].

Higher levels of homocysteine in both vegetarians and non-vegetarian in our study could be attributed to low folate intake in non-vegetarian and low vitamin B_{12} content in vegetarian population. In our study we also observed significant reduction in serum homocysteine level in both vegetarian and non-vegetarian of the patient population receiving folic acid treatment. Our findings lead to propose that vegetarians who do not supplement their diet with vitamin B_{12} tend to have elevated homocysteine levels; however a detailed study is recommended to confirm this.

Difference in LDL and TC of patients before and after treatment with folic acid were statistically significant in our study (Table 3). Recent studies suggest that elevated homocysteine levels are as important as high blood cholesterol levels and can operate independently [27].

Our findings have shown elevated levels of serum homocysteine before treatment with folic acid in all the hypertensive patients. Similar results were reported by Alina Atif et al [28]. Homocysteine may elevate blood pressure by causing arterial stiffness due to impaired vascular endothelial integrity and/or by reducing the efficiency of vasodilation. Homocysteine induced oxidative stress causes endothelial cell injury and reduces available nitric oxide, a potent vasodilator and therefore elevates blood pressure. A randomized study reported that DBP decreased 1.9 mmHg and SBP decreased 3.7 mmHg with 5 mg folic acid and pyridoxine in CVD patients[29]. Our study reconfirms that homocysteine lowering therapy reduced both SBP (4.36 mmHg) and DBP (3.72 mmHg) by folic acid alone.

Higher Hcy levels were reported people with vein clots, IHD, MI [27] and Ischemic stroke [30, 31]. In our study, participants suffering from angina, AMI, hypertension and stroke all showed significantly higher homocysteine when compared to controls (P<0.0001) (Table 5). The increased risk of stroke in patients with hyperhomocysteinemia is due to its possible role in the pathogenesis of atherosclerosis, its adverse effects on the endothelial surface. Additionally there is a complex interplay of the oxidative product of homocysteine with vascular smooth muscles, connective tissue, plasma lipoproteins, clotting factors and platelets [31]. Increased plasma Hcy leads to the formation of atheromatous changes, which ultimately cause MI [27].

Our finding was consistent with published data and confirm that reduction in homocysteine level will occur with folic acid but the degree of reduction varied in different studies [13, 32, 33]. In our study we have not attempted to examine the cause of homocysteine elevation. But folic acid supplementation given to CVD patients has really helped to reduce serum homocysteine level in all patients despite the presence of risk factors such as alcoholism, smoking, age and CVD.

CONCLUSION

The results of our study demonstrated reduction in Hcy, blood pressure, LDL and TC levels after an intake of folic acid 5 mg/day for five months. Hence it is recommended that all patients who present with hyperhomocysteinemia (HHC) be supplemented with folic acid and vitamin B_{12}. Patients irrespective of their diseases should all be screened for homocysteine status along with all other regular laboratory investigations. Screening for homocysteine in general public is advisable for early detection and protection from hypertension, hyperlipidemia, CVD, Alzheimer's disease etc.

Conflict of interests

The authors have declared that no conflict of interest exists.

REFERENCES

1. Mansoor Rastegarpanah *et al*. A new horizon in primary prevention of cardiovascular disease, can we prevent heart attack by "Heart Polypill"?. *Arch Iranian Med* 2008; 11 (3): 306-313.
2. Thomas Gazoiano A *et al.* Cardiovascular disease prevention with a multidrug regimen in the developing world: a cost effective analysis. *Lancet* 2006 August 19: 368 (9536): 679-686.
3. Wald N J, Law M R. A strategy to reduce cardiovascular disease by more than 80%. *BMJ* 2003; 326: 419-23.
4. Saeed sadeghian *et al*. Homocysteine, vitamin B_{12} and folate levels in premature coronary artery disease. *BMC* cardiovascular disorders 2006; 6: 38.

5. Kaul S *et al*. Homocysteine Hypothesis for Atherothrombotic Cardiovascular Disease – Not Validated. *JACC* 2006; 48: 914-23.
6. Skovby F. Inborn errors of metabolism causing homocysteinemia and related vascular involvement. *Haemostasis* 1989; 19 (1): 4-9.
7. Ubbink JB. The role of vitamins in the pathogenesis and treatment of hyperhomocyst(e)inaemia. *J Inherit Metab Dis* 1997; 20: 316-325.
8. Bejoy Baby *et al*. Elevated concentrations of plasma homocysteine in coronary artery disease patients with normal folate level. *J. Pharm. Sci. & Res.* 2009; 1 (2): 51-56.
9. Stanger O. Physiology of folic acid in health and disease. *Curr Drug Metab* 2002; 3: 211-23.
10. Noreen Sultan *et al*. Effect of folic acid supplementation on homocysteine level in postmenopausal women. *Abottabad* 2007; 19 (4):78-81.
11. Djuric D *et al*. Homocysteine, folic acid and coronary artery disease: Possible impact on prognosis and therapy. *Indian J Chest Dis Allied Sci* 2008; 50: 39-48.
12. Van Dijk RA *et al*. Long-term homocysteine-lowering treatment with folic acid plus pyridoxine is associated with decreased blood pressure but not with improved brachial artery endothelium dependent vasodilation or carotid artery stiffness: a 2-year, randomized, placebo-controlled trial. *Arterioscler Thromb Vasc Biol* 2001; 21: 2072-9.
13. Jalali F, Hajian-Tilaki K.O. Effect of Folic acid on Serum Homocysteine and Morbidity in Patients with Chronic Coronary Artery Disease. *Iranian Heart Journal* 2007; 8 (2): 44-50.
14. Wou KS, Chook P. Long term improvement in homocysteine levels and arterial endothelial function after 1 year folic acid supplementation. *The American Journal of Medicine* 2002; 112: 535 – 39.
15. Stanger O *et al*. Effects of folate treatment and homocysteine lowering on resistance vessel reactivity in atherosclerotic subjects. *J Pharmacol Exp Ther* 2002 Oct; 303(1):158–62.
16. Nygard O *et al*. Total plasma homocysteine and cardiovascular risk profile. The Hordaland Homocysteine Study. *JAMA* 1995; 274: 1526–33.
17. Brilakis ES *et al*. Lack of association between plasma homocysteine and angiographic coronary artery disease in the era of fortification of cereal grain flour with folic acid. *Atherosclerosis* 2002; 165: 375-381.
18. Scott CW *et al*. The Worldwide Smoking Epidemic: Council Reports. *JAMA* 1990; 24: 3312–18.
19. McCarty MF. Increased homocysteine associated with smoking, chronic inflammation and aging may reflect acute-phase induction of pyridoxal phosphatase activity. *Med Hypotheses* 2000; 55: 289–93.
20. O'callaghan P *et al*. Smoking and plasma homocysteine. *European heart journal* 2002; 23: 1580–1586.
21. Nygard O *et al*. Major lifestyle determinants of plasma total homocysteine distribution: the Hordaland Homocysteine Study. *Am J Clin Nutr* 1998; 67: 263–70.
22. El-Khairy L *et al*. Lifestyle and cardiovascular disease determinants of total cysteine in plasma: the Hordaland Homocysteine Study. *Am J Clin Nutr* 1999; 70: 1016–24.
23. Bleich S *et al*. Elevated homocysteine levels in alcohol withdrawal. *Alcohol and alcoholism* 2000; 35: 351-54.
24. Abraham R *et al*. Raised serum homocysteine levels in patients of coronary artery disease and the effect of vitamin B_{12} and folate on its concentration. *Indian journal of Clinical Biochemistry* 2006; 21 (1): 95-100.
25. Jayantee Kalita *et al*. A study of homocysteine level in North Indian subjects with special reference to their dietary habit. *European e-Journal of Clinical Nutrition and Metabolism* 2007; 2 (6): 116-119.
26. Misra A *et al*. Hyperhomocysteinaemia, and low intakes of folic acid and vitamin B12 in urban North India. *Eur.J. Nutr*. 2002; 41 (2): 68-77.
27. Yadav A. S *et al*. Relationship of plasma homocysteine with lipid profile parameters in ischemic heart disease. *Indian Journal of Clinical Biochemistry* 2006; 21 (1): 106-110
28. Alina Atif *et al*. Serum homocysteine concentrations in patients with Hypertension. *Pak J Physiol* 2008; 4 (1): 21-2.
29. Unhee Lim, Patricia Cassano A. Homocysteine and Blood Pressure in the Third National Health and Nutrition Examination Survey, 1988–1994. *Am J Epidemiol* 2002; 156: 1105–1113.
30. Loralie J *et al*. Hyperhomocyst(e)inemia and increased risk of venous thromboembolism. *Archives of Internal Medicine* 2000; 160: 961-964.
31. Narang A. P. S *et al*. Homocysteine – risk factor for ischemic stroke?. *Indian J Physiol Pharmacol* 2009; 53 (1): 34–38.

32. Wald DS *et al*. Randomized trial of folic acid supplementation and serum homocysteine. *Arch Intern Med* 2001 Mar; 161 (5): 697–700.
33. Clarke K, Armitage J. Vitamin supplements and cardiovascular risk: review of the randomized trials of homocysteine lowering vitamin supplement. *J Am Coll Cardiol* 2003; 141 (12): 2100 – 2104.

Table 1. Demographic, behavioral and nutritional variances of study groups

Parameter	Patients (n=50)	Controls (n=25)
Mean age in years	52.9±4.699(0.6645)	49 ± 2.404 (0.7601)
BMI (Kg/m^2)	27.282 ±2.331(0.6645)	24.25 ± 1.665 (0.5265)
Sex (%)		
Men	31 (62%)	15 (60%)
Women	19 (38%)	10 (40%)
Diet		
Vegetarian (%)	11 (22%)	5 (20%)
Non-vegetarian (%)	39 (78%)	20 (80%)
Smoking (%)	25 (50%)	5 (20%)
Non-smoking (%)	25 (50%)	20 (80%)
Alcoholics (%)	7 (14%)	3 (12%)
Non- alcoholics (%)	43 (86%)	22 (88%)

Table 2. Homocysteine, total cholesterol, triglycerides, HDL, LDL and Blood Pressure levels of CVD patient group and control group

Parameter	Patients (n=50)	Controls (n=25)	P value
Homocysteine (µmol/L)	22.91± 3.125	14.4 ± 1.39	<0.001
Total cholesterol (mg/dL)	217.5 ± 11.83	163.4 ± 24.21	<0.001
Triglycerides (mg/dL)	218.64± 16.78	180.6 ± 21.55	<0.001
HDL (mg/dL)	43.6± 4.16	43.9 ±3.64	0.0903
LDL (mg/dL)	130.64 ± 11.29	83.3 ± 23.37	<0.001
Systolic blood pressure (mmHg)	151.84 ± 5.02	132.2 ± 4.89	<0.001
Diastolic blood pressure (mmHg)	94.68 ± 3.145	87.04 ± 2.638	<0.001

Values are given as mean ± SD from 50 CVD patients and 25 controls. Statistical significance was considered as $p < 0.05$ at 95% confidence interval.

Table 3. Change in lipid profile and Blood Pressure on treatment with folic acid in CVD patient group

Parameter	Patients		P value
	Before treatment with folic acid	After treatment with folic acid	
TC (mg/dL)	217.5 ± 11.83	196.48 ± 13.84	0.0008
TG (mg/dL)	218.64± 16.78	201.44 ± 14.72	0.0709
HDL (mg/dL)	43.6± 4.16	45.8 ± 3.52	0.2515
LDL (mg/dL)	130.64 ± 11.29	110.44 ± 16.11	0.004
SBP (mmHg)	151.84 ± 5.02	147.48 ± 3.08	<0.0001
DB (mmHg)	94.68 ± 3.145	90.96 ± 1.059	<0.0001

Values are given as mean ± SD from 50 CVD patients and 25 controls. Statistical significance was considered as $p < 0.05$ at 95% confidence interval.

Table 4. Mean serum homocysteine levels with correlation to different age population in CVD patients and control subjects

Different age population	Homocysteine (µmol/ L)		P value
	Control subjects	CVD patients	
45-50	12.01 ± 2.162	18.688 ± 3.076	<0.0001
50-55	14.21 ± 2.644	19.364 ±3.100	<0.0001
55-60	15.27 ± 1.823	24.52 ± 3.240	<0.0001
60-65	15.46 ± 1.462	25.52 ± 2.942	<0.0001

Values are given as mean ± SD from Values are given as mean ± SD from 50 CVD patients and 25 controls. Statistical significance was considered as $p < 0.05$ at 95% confidence interval.

Table 5. Mean serum homocysteine levels with behavioral and clinical factors of CVD patient and control groups

Behavioral and clinical factors	Homocysteine (µmol/L)		P value
	Patients	Controls	
Men	23.21 ± 2.96 *	15.2 ± 1.62	<0.0001
Women	21.52 ± 2.70 *	13.6 ± 1.18	<0.0001
Vegetarian	24 ± 2.67 **	14.8 ± 1.64	<0.0001
Non-vegetarian	20.83 ± 3.07 **	14 ± 1.56	<0.0001
Smokers	24.5 ± 1.98 ***	16.2 ± 1.56	<0.0001
Non-smokers	20.32 ± 2.992 ***	12.6 ± 1.39	<0.0001
Alcoholics	25.17 ± 1.414 ****	14.9 ± 1.35	<0.0001
Non-alcoholics	20.66 ± 3.025****	13.9 ± 1.21	<0.0001
Hypertensives	22.92 ± 3.225	15.9 ± 1.68	<0.0001
Angina	22 ± 3.125	14.4 ± 1.39	<0.0001
AMI	24.66 ± 2.517	14.4 ±1.39	<0.0001
Stroke	21.2 ±0.7071	14.4 ± 1.39	<0.0001

Values are given as mean ± SD from 50 CVD patients and 25 controls. Statistical significance was considered as $P < 0.05$ at 95% confidence interval

* P = 0.1024 (Men Vs women in patient group)

** P = 0.0032 (Vegetarian Vs non-vegetarian in patient group)

***P< 0.0001 (Smokers Vs non-smokers in patient group)

****P = 0.0003 (Alcoholics Vs non-alcoholics in patient group)

Table 6. Change in serum homocysteine on treatment with folic acid in CVD patient group

Parameters	Homocysteine (µmol/L)		P value
	Before treatment with folic acid	After treatment with folic acid	
Men	**23.21 ± 2.96**	14.26 ± 2.68	<0.0001
Women	**21.52 ± 2.70**	15 ± 3.94	<0.0001
Vegetarians	**24 ± 2.67**	14.66 ± 3.14	<0.0001
Non-vegetarians	**20.83 ± 3.07**	15.07 ± 3.57	<0.0001
Smokers	**24.5 ± 1.98**	14 ± 2.76	<0.0001
Non-smokers	**20.32 ± 2.992**	15.27 ± 3.57	<0.0001
Alcoholics	**25.17 ± 1.414**	15.5 ± 2.64	<0.0001
Non-alcoholics	**20.66 ± 3.025**	14.38 ± 3.30	<0.0001
Hypertensives	**22.92 ± 3.225**	14.56 ± 1.39	<0.0001
Angina	**22 ± 3.125**	14.47 ± 2.85	<0.0001
AMI	**24.66 ± 2.517**	16.25 ± 4.86	<0.0001
Stroke	**21.2 ±0.7071**	12 ± 1.41	<0.0001

Values are given as mean ± SD from 50 CVD patients and 25 controls. Statistical significance was considered as $P < 0.05$ at 95% confidence interval.