"Should Hyperhomocysteinemia be ignored?" A case control prospective study to assess the magnitude of risk associated with hyperhomocysteinemia in chronic stable angina and the implications of Folic acid, B6 and B12 therapy.

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Abstract

**Background:** Results from prospective studies of serum homocysteine levels and ischemic heart disease (IHD) are inconclusive.

**Objective:** The objective of the present study was to determine the magnitude of risk associated with increased plasma homocysteine levels in the absence of other conventional risk factors in patients of chronic stable angina and to determine the extent of its reduction by folic acid, pyridoxine (B6) and B12 therapy.

**Research Design:** The study included 50 subjects of chronic stable angina with equal number of age and sex matched normal healthy individuals. The total plasma homocysteine, lipid profile, blood urea and serum creatinine levels were estimated in both the study groups.

**Results and Interpretations:** The mean plasma homocysteine concentration was higher in patients as compared to the control subjects and the difference was highly significant (p<0.01). A significant reduction in plasma homocysteine concentration (p<0.01).

**Conclusion:** Moderately high plasma homocysteine concentration is an independent but modifiable risk factor in the pathogenesis of coronary artery disease. Homocysteine can be lowered safely and effectively with folic acid and B-vitamin supplementation. The potential benefit of lowering homocysteine can have great implications for the primary and secondary prevention of ischemic heart disease.

**Key Words:** Homocysteine, Folic acid, Pyridoxine, Chronic stable angina, Cholesterol, Triglycerides, Very low density lipoprotein (VLDL), Low density Lipoprotein (LDL), High density lipoprotein (HDL)

**Introduction**

In the history of medicine, no other disease has equaled the medical, social, financial and emotional impact of coronary artery disease (CAD). Indians all over the world have the highest morbidity and mortality from coronary artery disease. The conventional risk factors like hypertension, insulin resistance, diabetes mellitus, abdominal obesity, physical inactivity, cigarette smoking and dyslipidemia are relatively low amongst Indians but the prevalence is more than two folds higher than the western population and this re-emphasizes the need for new guidelines for Indians. The link between high levels of serum homocysteine and atherosclerotic disease has been suspected since 1969 (1) but several unresolved questions remain. Homocysteine is a sulfur amino acid and a normal intermediate in methionine metabolism. Genetic factors, smoking, hypertension, serum creatinine, total cholesterol, protein and nutritional factors such as vitamin B6, B12 and folate deficiency determine serum total homocysteine (tHcy) concentrations.(3) As an emerging independent risk factor for cardiovascular disease homocysteine related research has sparked a vigorous debate over the past decade. It has been shown that elevated serum Hcy levels are associated with an increased risk of ischemic heart disease (IHD) and stroke (4,5,6). Also, higher Hcy concentrations in IHD or stroke patients than in controls has been reported (4, 7, 8). Some prospective and case-control studies with inconsistent results, some with highly significant results (8,9) and others with no association have been observed. (4,9,10). Therefore, an inconsistency exists among the prospective study results: some are negative, whereas others support the association with IHD, stroke, and other thrombotic events demonstrated in retrospective studies.

**Objective Of The Study:** In the light of the above mentioned facts, the objective of the present study was

1. To ascertain the levels of plasma homocysteine in patients of coronary artery disease and to compare them with those of age and sex matched normal
healthy individuals
2. To assess the critical levels of homocysteine that can cause CAD
3. To find the correlation of plasma homocysteine with other biochemical parameters and
4. To ascertain the effects of folic acid, B6 and B12 supplementation therapy on plasma homocysteine levels.

Research Design and Methods

A case control study was carried out including 50 patients suffering from chronic stable Angina, in the age range of 30-75 years, diagnosed on the basis of history, clinical symptoms, laboratory investigations, stress test (TMT), electrocardiogram and angiography. The patients had last episode of angina more than a year back and from then they were symptom less or without any episode of fresh ischemia. Chronic smokers and the patients with hypertension, renal failure, rheumatoid arthritis, hypothyroidism, diabetes mellitus, dyslipidemia, obesity and those taking drugs like theophylline, oral contraceptives and multivitamin were excluded from the study. 50 normal healthy volunteers, age and sex matched, from the same population, but without any history of any disease or family history of premature atherosclerosis were selected to serve as controls. The baseline examination included an interview on the history of smoking, alcohol intake, physical activity, dietary habits, use of medication, and history of chest pain suggestive of CHD. BMI was calculated as the weight in kilograms per the square of height in meters, and blood pressure was measured with the person in the sitting position after a 5-min rest. A patient was defined as having hypertension if systolic blood pressure was ≥160 mmHg, if diastolic pressure was ≥95 mmHg, or if the patient was receiving drug treatment for hypertension. The patients were screened for plasma homocysteine, lipid profile, renal profile and blood sugar levels. Plasma homocysteine levels were estimated by Enzyme Immuno Assay of Frantzen et al (11) All blood specimens were drawn at 8:00 a.m. after a 12-h fast. Samples were centrifuged within 1 hour and the sera frozen immediately at -20°C. Fasting plasma glucose was determined by the glucose oxidase method (Boehringer Mannheim, Mannheim, Germany). Serum lipid and lipoprotein cholesterol levels were measured in fresh serum samples. Serum total cholesterol and triglyceride levels were determined enzymatically (Boehringer Mannheim). Serum HDL cholesterol level was determined enzymatically after precipitation of LDLs and VLDLs with dextran sulfate MgCl2(12). Plasma creatinine level was determined by the Jaffe method (13). Serum urea was determined by an enzymatic method (urease- modified Berthelot reaction using kit provided by (BioMerieux/ France) (14). A quality control was well maintained. All the patients with Hyperhomocysteinemia were given folic acid 5mg/day, vitamin B12(50 mg/day) and vitamin B12(1mg/day) to be taken orally for 8 weeks, besides that the routine symptomatic treatment of angina was continued as such. After 8 weeks all the investigations, as were done before, were carried out to see the effect of supplementation therapy. Informed written consent was obtained from all participants.

Statistical Analyses: All statistical analyses were performed by using SPSS for Windows, version 10.0 (SPSS, Chicago, IL). Independent t-test were applied for finding the associations and correlations and comparing the biochemical parameters between the two groups. The results were shown as mean ± SD, and P < 0.05 was considered significant. Pearson correlation coefficients (r) were performed to assess the relationship of plasma homocysteine, serum creatinine and other lipid parameters.

Results and Discussion

The plasma homocysteine concentration in patients was found to be higher than the age matched control subjects (p<0.001). The levels of plasma homocysteine were found to be ranging between 9.7-36.5μM/L in comparison to 6.7-15.2μM/L in control subjects depicting only a moderate type of homocysteinemia in patients of CAD. Similar results have been reported by other studies (15,16).

On the basis of plasma homocysteine concentration the patients were distributed in to three groups (A, B and C) with values varying between 5-15 μM/L, >15-25μM/L and 25 μM/L respectively. Maximum number of patients (n=20) were found in group C (Table-1). The mean ± S.D. plasma homocysteine in patients was 21.43± 7.64μM/L in comparison to 10.3±2.88 μM/L of normal subjects and the difference in the values between these study groups was statistically highly significant (p<0.001).

The least value of plasma homocysteine in patients was observed to be 9.7 μM/L. Majority of the patients (n=44) were having plasma homocysteine >10 μM/L (Table 1), implying thereby that any value of plasma homocysteine >10 μM/L irrespective of age and sex of an individual is a risk factor for CAD and
homocysteine levels by suitable supplemen- tations.

The mechanism by which homocysteine increases the risk for IHD are yet not completely known. There is evidence that homocysteine might disturb the bioavailability of nitric oxide (NO), which would, at least in part, contribute to the pathophysiology of circulatory disor- ders in subjects with hyperhomocysteinaemia.(17)

There are 3 distinct autosomal-recessive inborn errors of metabolism in which homozygotes have very high serum homocysteine levels (about 10-50 times higher than the general population) and very high risk of premature cardiovascular disease: (1) cystathionine β -synthase deficiency, (2) 5,10-methylene tetrahydrofolate reductase deficiency, and (3) the cobalamin metabolic defects that result in impaired methionine synthase activity.(18)

Homozygotes for these 3 disorders have serum homocysteine levels about 3 times the population average and high risk of cardiovascular disease.(4). The only biochemical change in common among these 3 inborn errors of metabolism is a high homocysteine level; no other metabolite is consistently high or low in all 3. Given that cardiovascular disease is also common to all 3 genetic disorders, it follows that it is the homocysteine or a metabolite derived from it that is the cause of the IHD and not that homocysteine is merely a marker of some other cause.(19)

Another genetic defect, affecting about 10% of the population (homozygous for a thermo labile form of 5,10-methylene tetrahydrofolate reductase), also leads to moderately raised homocysteine levels and moderately increased risk of IHD (20)

A rise in plasma homocysteine concentration with increasing age was observed among normal subjects (Table II). At the same time, in patients, the concentration in patients was not found to be age dependent, instead the maximum concentration was observed in the younger age group <35 years(Table II). The relationship between serum homocysteine level and IHD seems to be stronger in younger persons than in older persons.(21)

The higher levels of plasma homocysteine in the normal elderly individuals (Table II) could be due to nutritional deficiencies or diminished activities of the enzymes responsible for the metabolism of homocysteine (19). Thus elderly normal individuals with higher homocysteine levels are more at risk for CAD, in the absence of other conventional risk factors.
normal individuals were observed to be 191.75±35.62 mg/dL and 191.07±23.46 mg/dL respectively. Similarly, the values of serum triglycerides were found to be 160.89± 8.72 mg/dL and 148.04± 56.72 mg/dL. Serum VLDLc values were 32.17± 17.44 mg/dL and 29.60± 11.34 mg/dL and that of serum LDLc were 117.36± 26.5 mg/dL, and 109.42± 22.17 mg/dL amongst patients and control subjects respectively. The difference between the mean levels of serum total cholesterol, serum triglycerides, serum VLDLc and serum LDLc was observed to be statistically insignificant (p>0.05) in each case. The mean ± SD HDLc levels in patients and control subjects were 41.21± 7.54 mg/dL and 52.05± 6.41 mg/dL respectively. Although the levels amongst patients were relatively on the lower side, yet they were well within the normal range. The difference between the two groups was statistically highly significant (p< 0.001). The ratio of total cholesterol: HDLc amongst patients and control subjects was 4.7± 0.88 and 3.73±0.69 respectively. The difference between the two was although highly significant (p<0.001), yet the values were within the normal limits and this could be attributed to relatively lower level of HDLc in patient’s group. The ratio of LDLc: HDLc also followed the similar trend with values amongst patients and control subjects of 2.88± 0.65 and 2.14±0.53 respectively. Statistically these were highly significant variations (p<0.001) but were still within the normal range.

Blood urea was found to be normal amongst all the patients (Figure II). Serum creatinine levels in patients showed certain variations. The mean ± SD of serum creatinine levels in patients and control subjects were 1.061± 0.19 mg/dL and 0.884±0.16 mg/dL respectively. The levels were normal but again the difference was statistically highly significant (p< 0.001) Figure II. Evaluation of serum creatinine levels of various groups A, B and C revealed a parallel trend to that of plasma homocysteine. More the plasma homocysteine more were the serum creatinine levels from group A to B and then to C. A positive co-efficient of correlation (0.676) was observed between the serum creatinine concentration and plasma homocysteine levels (Table IV).

Highly significant reduction (45%) in plasma homocysteine concentration (p< 0.001) (Table I) was achieved after folic acid, B6 and B12 therapy. A folic acid supplement of 0.4 mg/d has been shown by Ward and colleagues (25, 26) to reduce average homocysteine levels in middle-aged patients by 1.9 µmol/L, it is equivalent to a 10% reduction in IHD mortality (27, 28). The levels of lipid parameters were also affected by folic acid therapy. There was rise in the serum total cholesterol from pretreatment 190.07± 35.62 mg/dL to 206±31.6 mg/dl after the treatment (Figure-I). The rise was statistically significant but the values never exceeded the normal limits. Serum triglyceride level approached 173.2± 73.8 mg/dL from that of 160.9±87.21 mg/dL of pretreatment value. VLDLc followed the same trend with rise in the levels from 32.17±17.44 mg/dL to 34.65±14.76 mg/dL. The level of LDLc was also increased with values reaching from 117.3 ±26.5 mg/dL to 124.0 ±14.76 mg/dl. All these variations (serum triglycerides, VLDLc and LDLc) were statistically insignificant (p>0.10) and the values also remained within the normal limits. HDLc levels were improved after the therapy. A highly significant rise was observed after the therapy (p<0.001) with levels rising from 41.21 ± 7.54 mg/dL to 48.26± 6.87 mg/dl.

The ratios of total cholesterol: HDLc and LDLc: HDLc showed the insignificant variations (p>0.05) and (p>0.10) respectively.

Serum creatinine levels were reduced after folate therapy from 1.06±0.19 mg/dl to 0.83±0.23 mg/dL. The difference between the pre and post treatment levels was statistically highly significant (p<0.001) (Figure-III). Maximum reduction was observed in group C of that of maximum plasma concentration. A positive coefficient of correlation was observed between plasma homocysteine and creatinine concentration(29) both before and after treatment (Table III). These findings reinforce the fact that serum creatinine content is dependent on the plasma homocysteine content and its metabolism in the biological system. Blood urea levels were not affected by the folate therapy.

Since the patients were having normal lipid profile(Figure-II) and a negative coefficient of correlation was observed between plasma homocysteine and lipid parameters (30) (Table III), the other conventional risk factors like smoking hypertension, diabetes mellitus, obesity etc were eliminated during selection of the subjects for study, hence elevated plasma homocysteine could only be the possible risk factor for CAD amongst such patients. Thus this proves the fact that the elevated plasma homocysteine could be an independent risk factor for CAD.

**Conclusion and Recommendations**

Any value of plasma homocysteine 10 µM/L even in
younger age group irrespective of gender is an independent risk factor for CAD. A supplementation of folic acid, B6, B12 in the dose of 5 mg/day, 50mg/day and 1mg/day respectively can bring about significant reduction (35-45%) in the level of plasma homocysteine (26). Folic acid could be an inexpensive and effective measure in the prevention and treatment of coronary heart disease in the future days to come.

Abbreviations

IHD - Ischemic heart disease
CAD - coronary artery disease
tHcy - serum total homocysteine
VLDL - Very low density lipoprotein
LDL - Low density Lipoprotein
HDL - High density lipoprotein

Acknowledgement(s)

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References

Illustrations

Illustration 1

Table 1: Comparison of Plasma Homocysteine levels in patients of Angina before and after folate, B6 and B12 supplementation

<table>
<thead>
<tr>
<th>S.No</th>
<th>Subjects</th>
<th>Group</th>
<th>Number of Subjects</th>
<th>Plasma Homocysteine concentration(µM/L) Pre-Treatment</th>
<th>Plasma Homocysteine concentration(µM/L) Post Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Range</td>
<td>Mean ±S.D.</td>
</tr>
<tr>
<td>1.</td>
<td>Normal</td>
<td>-</td>
<td>50</td>
<td>6.3-15.2</td>
<td>10.3±2.882</td>
</tr>
<tr>
<td>2.</td>
<td>Patients</td>
<td>-</td>
<td>50</td>
<td>9.7-36.5</td>
<td>21.43±7.64</td>
</tr>
<tr>
<td>i)</td>
<td>A</td>
<td>16</td>
<td>9.7-14.9</td>
<td>12.53±1.965</td>
<td>0.694</td>
</tr>
<tr>
<td>II)</td>
<td>B</td>
<td>14</td>
<td>16.1-2 3.8</td>
<td>20.52±2.74</td>
<td>1.03</td>
</tr>
<tr>
<td>III)</td>
<td>C</td>
<td>20</td>
<td>26.8-3 6.5</td>
<td>29.19±3.12</td>
<td>0.98</td>
</tr>
</tbody>
</table>

Pre-Post Treatment (Patients) t = 10.382 p< 0.001 (Highly significant)
(Group A) t = 5.643 p< 0.001 (Highly significant)
(Group B) t = 15.679 p< 0.001 (Highly significant)
(Group C) t = 15.032 p< 0.001 (Highly significant)
Illustration 2

Table 2: Plasma homocysteine concentration in different age groups of normal healthy individuals and patients of Angina

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Age Group(Years)</th>
<th>Plasma Homocysteine concentration (µM/L)</th>
<th>In Normal Individuals</th>
<th>In Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. of Subjects</td>
<td>Mean ± S.D.</td>
<td>No. of Subjects</td>
</tr>
<tr>
<td>I</td>
<td>35-45</td>
<td>12</td>
<td>8.2±2.750</td>
<td>10</td>
</tr>
<tr>
<td>II</td>
<td>46-55</td>
<td>22</td>
<td>10.17±2.579</td>
<td>22</td>
</tr>
<tr>
<td>III</td>
<td>&gt;55</td>
<td>16</td>
<td>12.03±2.525</td>
<td>18</td>
</tr>
<tr>
<td>Total</td>
<td>35-76</td>
<td>50</td>
<td>10.37±2.82</td>
<td>50</td>
</tr>
</tbody>
</table>
Table 3: Variables correlated with plasma homocysteine concentration

<table>
<thead>
<tr>
<th>S. No</th>
<th>Biochemical Parameters</th>
<th>Control</th>
<th>Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pre Treatment</td>
<td>P</td>
</tr>
<tr>
<td>1.</td>
<td>Serum Creatinine</td>
<td>0.176</td>
<td>0.676</td>
</tr>
<tr>
<td>2.</td>
<td>Serum Cholesterol</td>
<td>-0.2210</td>
<td>-0.4241</td>
</tr>
<tr>
<td>3.</td>
<td>Serum Triglycerides</td>
<td>0.1318</td>
<td>-0.4142</td>
</tr>
<tr>
<td>4.</td>
<td>Serum VLDL c</td>
<td>0.1322</td>
<td>-0.4142</td>
</tr>
<tr>
<td>5.</td>
<td>Serum LDLc</td>
<td>-0.2089</td>
<td>-0.1833</td>
</tr>
<tr>
<td>6.</td>
<td>Serum HDLc</td>
<td>-0.3018</td>
<td>-0.4009</td>
</tr>
</tbody>
</table>
Illustration 4

Fig 1: Mean Plasma Homocysteine levels in males and females under study
Illustration 5

Figure 2: Showing complete Lipid profile of normal control subjects and patients of Angina before and after folate, B6 and B12 supplementation
Illustration 6

Figure 3: Comparison of Serum Creatinine levels in patients of Angina before and after Folate, B6 and B12 supplementation
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