Homocysteine and Coronary Artery Disease

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Indians are more prone to premature coronary artery disease. High serum homocysteine levels have been recognized as one of the novel risk factors of coronary artery disease. Hyperhomocysteinemia has been associated with increased risk of atherosclerosis and myocardial infarction by a number of prospective case-control studies. A variety of genetic mutations, nutritional deficiencies including low intakes of folate, vitamin B₁₂ and B₉, disease states, and drugs can elevate homocysteine concentrations. It has been suggested that taking combination of vitamin B₁₂, vitamin B₁₃, folic acid, trimethyl glycine or simply folate may be used to bring down homocysteine levels. This article reviews the biochemistry, homocysteine metabolism, pathogenesis, and etiology of hyperhomocysteinemia, along with its association with coronary artery disease.

Homocysteine referred to as ‘cholesterol of the 90s’, is a sulphur containing amino acid that is formed during intracellular metabolism of an essential amino acid, methionine, which is taken into body via animal-derived food. McCully first linked elevated plasma homocysteine (hcy) concentrations with vascular disease clinically in patients with homocysteinuria. The prevalence of hyperhomocysteinemia has been estimated to be 5% in the general population and 13-47% among patients with symptomatic atherosclerotic vascular disease. However, these estimates are based on a cut-off above the 90th or 95th percentile of the distribution of hcy in the general population. It was found that for a 5 μmol/l increment in fasting plasma hcy, the odds ratio of coronary artery disease was 1.2 in Indian Asians, and 1.3 in Europeans. Hcy is up to 40 times more predictive than cholesterol in assessing coronary artery disease (CAD) risk. It has been reported that hyperhomocysteinemia is caused by deficiency of folate, vitamin B₁₂, vitamin B₉, and thus it has been suggested that taking combination of these vitamins may be used to bring down hcy levels. In this review we have discussed hcy in specific relation to CAD.

BIOCHEMISTRY OF HOMOCYSTEINE

Hcy is present in plasma in 4 forms. Majority of hcy (70-80%) forms disulphide bonds with proteins especially albumin to form so-called protein bound hcy. About 20-30 % dimerises with itself and 1 % circulates as free thiol. It can combine with other thiols including cysteine. Total hcy (t-hcy) refers to combined pool of all 4 forms. Since food contains little or no free hcy, nearly all of this non-protein forming sulphur amino acid is produced in body as an intermediate metabolic product of methionine and dietary hcy does not appear to affect the plasma hcy levels. Hcy in body is rapidly auto-oxidized in plasma to its disulfides and ultimately to hcy thiolactone.

Metabolism of Homocysteine:

Hcy is generated by the catabolism of methionine. This occurs by enzymatic condensation with adenosine tri-phosphate (ATP) to form S-adenosylmethionine and hydrolysis to release adenosine and hcy. Hcy in body is detoxified in two ways, under conditions of low protein intake the remethylation pathway may regenerate amounts of methion-
ine, a reaction catalyzed by methyltetrahydrofolate homocysteine methyltransferase (methionine synthase). This enzymatic reaction requires methyltetrahydrofolate as the methyl donor, which is generated by the enzyme methylenetetrahydrofolate reductase (MTHFR), and is also dependent on the co-factor methylcobalamin, a form of vitamin B₁₂. An additional minor remethylation mechanism is catalyzed by betaine homocysteine methyltransferase and utilizes betaine as a methyl donor to form N,N-dimethylglycine and methionine. Another potent remethylation agent is trimethyl glycine (TMG). This pathway requires folic acid, vitamin B₁₂, zinc and TMG. Choline is another methyl donor that helps to lower elevated hcy levels. This conversion doesn’t require cofactors. However, choline only enhances remethylation in the liver and kidney.

Trans-sulfuration is the other pathway responsible for the catabolism of major hcy via a pyridoxal-5'-phosphate (vitamin B₆)-dependent condensation with serine to form cystathionine, catalyzed by cystathionine B-synthase (CBS). Cystathionine is in turn hydrolyzed to cysteine and ß-ketobutyrate by ß-cystathionase. This reaction pathway converting hcy to cysteine is referred to as trans-sulfuration pathway. In the presence of excess methionine, trans-sulfuration is favored, with up-regulation of CBS and down-regulation of the remethylation pathway. Thus the net flow of carbon and sulfur from methionine is to cysteine, which is then oxidatively catabolized to sulfate and carbon dioxide.

DETERMINANTS OF HOMOCYSTEINE CONCENTRATIONS

Apart from CAD, hcy levels may increase or decrease in various other conditions. Various factors may determine plasma hcy concentrations (Table 1). In our study we found that hcy levels decreased as the age increased. It was highest in the youngest age group (30-40 y) and it decreased as the age increased. It was lowest in the highest age group (60-70 y) of patients. A large cohort of subjects from the Framingham Study indicated that folate, vitamin B₆, and vitamin B₁₂ are important determinants of plasma hcy concentrations in a healthy population. Wilcken and Gupta made the initial observations that hcy concentrations were elevated in patients with impaired renal function. In a prospective British Regional Heart Study a graded linear association between hcy and risk of stroke was observed. In a study by Clarke et al., patients with hyperhomocysteinemia have a higher odds ratio of developing all types of vascular disease, including peripheral vascular disease. Wilcken et al. made the first observation of a rising hcy concentration after renal transplantation in 1981. The mechanism of hyperhomocysteinemia in renal transplant patients may be related to renal impairment and folate resistance. A study carried out in Mexican population showed that there is a genetic factor with higher incidence of a TT homozygotic mutation of the MTHFR that increases homocysteine because of an altered folate metabolism. Many drugs may influence the hcy levels. Benzafibrate and fenofibrate may also elevate hcy concentrations perhaps by altering renal function. Niacin and theophylline, by causing a deficiency of vitamin B₁₂, raise hcy levels. Tamoxifen, used as an adjuvant therapy for breast cancer, has been shown to reduce hcy concentrations in postmenopausal breast cancer patients and healthy postmenopausal women.

DECREASING HOMOCYSTEINE LEVELS:

Dietary modifications including restricting methionine intake and/or increased consumption of fruits and leafy green vegetables can help lower the hcy levels by increasing the folate of diet. This can be tried as first step. However, most studies failed to show effectiveness of non-fortified, self-selected prescribed diets. Consequently, patients should repeat the t-hcy analysis after one month on the prescribed diet. If reduction in t-hcy is not achieved, daily supplementation including ingestion of combination of vitamin B₁₂, vitamin B₁₂, folic acid, TMG or simply folate may be used to bring down hcy levels. Combination of vitamin C and vitamin E may also act as antidote to hcy.

INDICATIONS FOR HCY DETERMINATION-A PROVISIONAL GUIDELINE

At present, utilization guidelines do not exist to direct clinicians or biochemists for hcy assays or for genetic analysis. Until further data are obtained, such guidelines will likely be based on patterns of practice. However, there are sufficient data to allow for the delineation of four major groups for whom testing is likely to be of more good than harm (Table 2).

EVIDENCES OF ASSOCIATION BETWEEN HYPERHOMOCYSTEINEMIA AND CAD

Various clinical studies have suggested that elevated hcy is a risk factor for atherosclerotic vascular disease and for arterial and venous thromboembolism. McCully described the correlation between hcy and CAD in two children with extensive arterial thrombosis and atherosclerosis having severe homocysteinemia. Various case-control studies have been robust in confirming the association between
### TABLE 1: FACTORS AFFECTING HOMOCYSTEINE CONCENTRATIONS

**Primary factors:**

- **Inherited enzyme deficiencies:**
  - Cystathione Beta-synthase deficiency
  - $C_{677}T$ mutation of methylene tetrahydrofolate reductase.
  - Methionine synthase deficiency
  - Methylene tetrahydrofolate homocysteine methyl transferase deficiency.

- **Acquired or secondary factors:**
  - **Physiological:**
    - Age
    - Sex
    - Diet and Lifestyle-Smoking, Alcohol
    - Menopause
  - **Nutritional Deficiency:**
    - Folic acid deficiency
    - Cobalamin (vitamin $B_{12}$) deficiency
    - Pyridoxine (vitamin $B_{6}$) deficiency
  - **Pathological:**
    - Renal disease
    - Renal transplantation
    - Post stroke
    - Severe psoriasis
    - Malignancies-breast, ovary, pancreas, acute lymphoblastic leukemia
    - Hypothyroidism
    - Pernicious anemia
    - Connective tissue disorders (rheumatoid arthritis and systemic lupus erythematosus)
    - Hepatic impairment
  - **Medications:**
    - Increased levels:
      - Methotrexate
      - Anticonvulsants (phenytoin, carbamazepine)
      - L-Dopa
      - Niacin and theophylline
      - Androgens
      - Cyclosporin, azathioprine
      - Thiazide diuretics
    - Decreased levels:
      - Penicillamine
      - N-Acetylcysteine
      - Betaine
      - Oral contraceptives
hyperhomocysteinemia and CAD (Table 3). There are however few studies that show no association between hyperhomocysteinemia and CAD (Table 3). Results from our study are consistent with the previous studies. We found a clear association between hyperhomocysteinemia and CAD more strong association in younger Indians12.

HOMOCYSTEINE-PATHOGENESIS IN OCCLUSIVE ATHEROSCLEROSIS

The possible mechanism of hyperhomocysteinemia-related vascular injury is still a subject of research. Numerous studies have explored sites of adverse influence of hcy, including the endothelial surface, vascular smooth muscle cells, connective tissue, interactions with plasma lipoproteins, clotting factors and platelets.

Endothelial effects:

Various experimental as well as clinical data suggest that the proatherogenic conditions associated with hyperhomocysteinemia results from endothelial dysfunction and injury followed by generation of various reactive oxygen species (ROS), platelet activation and thrombus formation. Pathophysiological concentrations of hcy induce increased adhesion between neutrophils and endothelial cells. This results in neutrophil migration across the endothelium, with concurrent damage and detachment of endothelial cells. Nitric oxide (NO) interacts with biological thiols, such as hcy, forming S-nitrosothiols or S-nitrosocysteine39. S-Nitrosohomocysteine has vasodilatory and antiplatelet actions39. It does not support the generation of hydrogen peroxide and does not undergo conversion to thiolactone. High concentrations of hcy impair generation of NO by endothelial cells and formation of protective S-nitrosocysteine may be impaired because of a decreased bioavailability of NO39. Other perturbations of endothelial function by hcy include enhanced neutrophil adhesion and migration across the endothelial barrier37, interference with transcription fac-

TABLE 2: SUGGESTED GUIDELINES FOR DETERMINATION OF TOTAL HOMOCYSTEINE

<table>
<thead>
<tr>
<th>Group 1: Evidence for vitamin deficiency</th>
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<tbody>
<tr>
<td>• Signs of B12 or folate deficiency with normal serum vitamin levels.</td>
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<tr>
<td>• Aberrant serum vitamin levels but apparently normal clinical status.</td>
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<tr>
<th>Group 2: Hyperhomocysteinemia in vaso-occlusive disease</th>
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<tbody>
<tr>
<td>• Strong family history, without other explanatory risk factors, for:</td>
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<tr>
<td>• Myocardial infarction</td>
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<tr>
<td>• Peripheral arterial disease</td>
</tr>
<tr>
<td>• Stroke</td>
</tr>
<tr>
<td>• Recurrent pulmonary embolism</td>
</tr>
<tr>
<td>• Venous thrombosis</td>
</tr>
<tr>
<td>• Conditions noted above, but in patients &lt; 35 years</td>
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<thead>
<tr>
<th>Group 3: Other high risk groups</th>
</tr>
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<tbody>
<tr>
<td>• Chronic renal failure, transplant recipients</td>
</tr>
<tr>
<td>• Parents of neural defect infants</td>
</tr>
<tr>
<td>• Patients on metabolic diets</td>
</tr>
<tr>
<td>• Chronic renal failure, transplant recipients</td>
</tr>
<tr>
<td>• Patients with cancer</td>
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</table>

<table>
<thead>
<tr>
<th>Group 4: Evidence of homocystinuria</th>
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</thead>
<tbody>
<tr>
<td>• Increased urinary hcy</td>
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<tr>
<td>• Dolichostenomelia (marfanoid habitus)</td>
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<tr>
<td>• Ocular lens dislocation, otherwise unexplained</td>
</tr>
<tr>
<td>• Other typical features</td>
</tr>
<tr>
<td>• Early vaso-occlusive disease</td>
</tr>
<tr>
<td>• Pneumothorax, osteopenia</td>
</tr>
</tbody>
</table>

Fig.1: Metabolism of homocysteine.
tors such as NFkB, and folding of glycoproteins in the endoplasmic reticulum. Hcy is rapidly auto oxidized to form potent ROS are produced during the auto oxidation of hcy. This generation of ROS induces endothelial dysfunction. Harker and colleagues have proposed that hcy-induced endothelial cell injury mediated by hydrogen peroxide exposes the underlying matrix and smooth muscle cells that in turn proliferate and promote the activation of platelets and leukocytes. Superoxide-dependant formation of the hydroxyl radical has been shown to initiate lipid peroxidation, an effect that occurs at the level of endothelial plasma membrane and within lipoprotein particles. Hcy auto-oxidation has been shown to support the oxidation of low-density lipoprotein through the generation of the superoxide anion radical. Hcy thiolactone, which is a highly reactive byproduct of hcy oxidation, combines with LDL to form aggregates that are taken up by macrophages and converted into the foam cell and incorporated in plaque development. It has been also suggested that hcy thiolactone impairs oxidative phosphorylation and promotes the proliferation and fibrosis of smooth muscles. Hcy also increases the formation of highly atherogenic oxycholesterols.

**Thrombosis:**

Hcy has been found to increase production and activation of procoagulant factors and inactivation of anticoagulant substance. High concentrations (300 µM) of hcy induce endothelial cell tissue factor expression and activity in vitro, which may initiate coagulation. Hcy indirectly increases activation of the procoagulant endothelial cell factor V and inactivates the anticoagulant substance, protein C and thrombomodulin. In addition, hcy stimulates platelet generation of the prostaglandin thromboxane A2, which is a vasoconstrictor and pro-aggregant. Furthermore, physiologic concentrations of hcy (8 µM) promote binding of lipoprotein (a) to fibrin, thereby preventing plasminogen activation and fibrinolysis. Recent data shows that hcy can directly block the tissue plasminogen activator binding domain of annexin II, which would be expected to inhibit thrombolysis and thereby promote thrombosis.

**Vascular Growth:**

Hcy stimulates growth promoting signal transduction pathways such as MAP Kinase in vascular smooth muscle.

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**TABLE 3: PROSPECTIVE STUDIES OF THE REALTIONSHP BETWEEN HOMOCYSTEINE AND THE INCIDENCE OF CORONARY ARTERY DISEASE**

<table>
<thead>
<tr>
<th>Authors</th>
<th>Study population</th>
<th>Association or not</th>
<th>N</th>
<th>Year</th>
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<tbody>
<tr>
<td>Clarke et al.</td>
<td>Ireland</td>
<td>Yes</td>
<td>175</td>
<td>1991</td>
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<td>Araki et al.</td>
<td>Japanese</td>
<td>Yes</td>
<td>226</td>
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<td>Perry et al.</td>
<td>US</td>
<td>Yes</td>
<td>5661</td>
<td>1995</td>
</tr>
<tr>
<td>Petri et al.</td>
<td>US</td>
<td>Yes</td>
<td>337</td>
<td>1996</td>
</tr>
<tr>
<td>Chasan-Taber et al.</td>
<td>US</td>
<td>No</td>
<td>14,916</td>
<td>1996</td>
</tr>
<tr>
<td>Ridker et al.</td>
<td>US Physicians</td>
<td>Yes</td>
<td>14,916</td>
<td>1997</td>
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<td>Bostom et al.</td>
<td>Framingham cohort</td>
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<td>1999</td>
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<tr>
<td>Nahalawi et al.</td>
<td>US</td>
<td>No</td>
<td>160</td>
<td>1999</td>
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<tr>
<td>Selhub et al.</td>
<td>US</td>
<td>Yes</td>
<td>587</td>
<td>1993</td>
</tr>
<tr>
<td>Evans et al.</td>
<td>US</td>
<td>No</td>
<td>712</td>
<td>1997</td>
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<td>Deepa et al.</td>
<td>Indian</td>
<td>No</td>
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<td>Gupta et al.</td>
<td>Indian</td>
<td>Yes</td>
<td>110</td>
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<td>Guo et al.</td>
<td>Chinese</td>
<td>Yes</td>
<td>89</td>
<td>2003</td>
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<td>Matetzky et al.</td>
<td>Israel</td>
<td>Yes</td>
<td>157</td>
<td>2003</td>
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<tr>
<td>Puri et al.</td>
<td>Indian</td>
<td>Yes</td>
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<tr>
<td>Bozkurt et al.</td>
<td>Turkey</td>
<td>Yes</td>
<td>341</td>
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cells. In addition, hcy increases the mitogenic response of platelet-derived growth factor on vascular smooth muscle cells four fold, possibly by disturbing the activity of antioxidant enzymes. Finally, hcy also stimulates plasma and aortic cycling dependent kinase, which may contribute to vascular smooth muscle growth.

CONCLUSIONS

Hyperhomocysteinemia is one of the major and independent risk factors for the CAD. Low serum folate levels are associated with hyperhomocysteinemia. Administration of low doses of folic acid, vitamin B6 and vitamin B12 keeps a check on high hcy levels in plasma. So here it is not irrelevant to say that dietary supplementation of such vitamins is both rational and appealing for lowering hcy levels in patients with hyperhomocysteinemia.

FUTURE PROSPECTS

Both diabetes and hyperhomocysteinemia are strong risk factors for CAD. In spite of higher prevalence of CAD compared with non-diabetic, the mean plasma hcy concentration is usually low or normal in insulin-dependent diabetes mellitus (IDDM) and NIDDM patients, except when nephropathy or impaired renal clearance is present. In contrast, Hoffman et al. found elevated hcy in NIDDM patients, but in the study vast majority of diabetic patients with fasting elevated hcy presented nephropathy. In our study we also found that there is no synergistic relationship between the two rather diabetes decreases hcy levels. Decrease in hcy level in diabetics may be due to glomerular hyper filtration in diabetes. The precise mechanism is yet to be evaluated. Reduction in hcy may improve endothelial function. Woo et al. showed improved endothelium-dependent vasodilatation after folic acid supplementation in patients with hyperhomocysteinemia. Whether this improvement is due to the hcy-lowering effect of folic acid or is due to other properties of folic acid is unclear. More prospective studies are thus required to evaluate the exact mechanism of action. Homocysteine-lowering therapy with folic acid, vitamin B12, and vitamin B6 significantly decreases the incidence of major adverse events after percutaneous coronary intervention. Whether the therapy corresponds with decreased risk of coronary events is unknown.

REFERENCES