HYPERHOMOCYSTEINEMIA: AN EMERGING RISK FACTOR FOR CARDIOVASCULAR DISEASE

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INTRODUCTION

Hyperhomocysteinemia was first described in 1962 in mentally retarded children (1). Subsequently in 1969, McCully made the clinical observation linking elevated plasma homocysteine concentrations with vascular disease (2). Later in 1976, Wilcken and Wilcken reported that patients with coronary artery disease (CAD) frequently have abnormal homocysteine metabolism (3). Recent studies are now confirming McCully's work of more than 30 years ago. Mild to moderate elevations of plasma homocysteine levels have been associated with cardiovascular diseases, including ischemic heart disease, cerebrovascular disorders and ischemic disorders of the lower limbs.

Multiple case control studies have demonstrated that the homocysteine level is higher among patients with proven ischemic heart disease and premature coronary artery disease as compared to a clinically healthy population (4). Selhub *et al* (5) have demonstrated that the prevalence of carotid artery stenosis increases with increasing plasma concentrations of homocysteine. A cross sectional study of 1041 elderly subjects in the Framingham Heart Study, found a strong association between elevated homocysteine concentrations and occlusive vascular disease. Boushey *et al* (6) estimated that 10 percent of the risk of coronary heart disease in the general population is attributable to homocysteine.

Chambers *et al* have reported that elevated plasma homocysteine concentrations is a risk factor for coronary heart disease, independent of conventional

risk factors including diabetes and metabolic disturbances associated with insulin resistance(7).

Population studies show that plasma homocysteine concentrations are higher in Indian Asian overseas, compared to the North Americans and European Whites (7, 8). Based upon the analytical relationship between homocysteine and coronary heart disease increments of this magnitude may correspond to a 30% increase in population at risk of coronary heart disease. These observations suggests that homocysteine may be a major determinant of increased coronary heart disease risk amongst Indian Asians the World over.

On the basis of recent retrospective, prospective and epidemiological studies from Europe, United States and Canada, it is now widely accepted that mild hyperhomocysteinemia is an independent risk factor for cardiovascular disease even in the presence of other traditional risk factors. Evidence from these studies have shown the association between homocysteine and cardiovascular disease in both men and women. In this review, we will evaluate the evidence of a relation between elevated plasma homocysteine concentrations and the vascular disease.

HOMOCYSTEINE METABOLISM

Homocysteine is a naturally occurring sulfur containing amino acid derived from the metabolism of dietary methionine. It has three main metabolic fates. It can be remethylated to methionine in the presence of vitamin B12 and folic acid. This is known as the remethylation cycle. Secondly, it can be sulfo- conjugated with serine to form cysteine in a series of enzymatic reactions, which require vitamin B6 as a cofactor. This is known as the Transsulfuration pathway. The third fate of homocysteine is its export into the extracellular medium which may reflect an imbalance between homocysteine production and its metabolism [9].

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Under normal conditions, methionine is derived from food protein. The average daily methionine intake is about 2 grams. To smoothly run the methionine transformation, body requires a sufficient supply of vitamin B6, B12 and folic acid. If there is a lack of these vitamins and a high intake of protein, eg., excessive meat intake, the intermediary product, homocysteine, starts to accumulate leading on to hyperhomocysteinemia.

ESTIMATION OF PLASMA HOMOCYSTEINE

Homocysteine is present in plasma in four forms: 70 – 80 % is disulfide bound to plasma proteins, chiefly albumin; about 1 % circulates as the free thiol; and the remaining 20-30% combines with itself to form the dimer homocysteine or with other thiols, including cysteine, with which it forms the homocysteine – cysteine mixed disulfide. The term "total plasma homocysteine" (t Hcy) refers to all the four forms of homocysteine.

Homocysteine is measured from a blood sample taken after a twelve hour fast. tHcy in serum or plasma is stable for weeks at 0 to 2 °C and for years when kept frozen at – 20 °C [10]. A normal tHcy among fasting individuals is between 5 to 15 m mol/L [11, 12]. Higher fasting levels are classified as moderate (>15 to 30 m mol/L), intermediate (> 30 to 100 mmol/L) and severe (> 100 m mol/L) hyperhomocysteinemia. values reaching as high as 500 mmol/L in patients with homocystinuria (13).

An oral dose of methionine (100 mg per kilogram of body weight) can be given to persons with suspected hyperhomocysteinemia who have normal homocysteine concentrations during fasting. This methionine load test measures tHcy levels before and between four and eight hours after the intake of methionine. Hyperhomocysteinemia is considered to be present in tHcy level after the methionine challenge is more than 2 S.D. above the mean [14]. This test may uncover 39 percent of subjects with homocysteine related cardiovascular disease risk but with normal basal tHcy levels (15).

Plasma tHcy levels are best estimated by using either the fluorescence polarization immunoassay (FPIA), enzyme linked immunosorbent assay (ELISA) or high pressure liquid chromatography (HPLC) or gas chromatography – mass spectrometry (GC-MS).

CAUSES OF HYPERHOMOCYSTEINEMIA

The causes of hyperhomocysteinemia can be divided into primary and secondary (Table 1). The primary causes of hyperhomocysteinemia include the genetic defects in the enzymes involved in homocysteine metabolism, whereas the secondary causes include life style factors, nutritional deficiencies in vitamin cofactors, chronic diseases and drugs. Cystathionine β synthase deficiency is the most common genetic cause of severe hyperhomocysteinemia. It occurs 1 in 1,00,000 live births. It is an inherited autosomal recessive trait. The clinical manifestations include ectopia lentis, skeletal deformities, mental retardation, thromboembolism and severe premature atherosclerosis (16). Heterozygous form about 1 in 150 population and their plasma homocysteine concentrations is in the range of 20 to 40 m mol / L. The most common enzyme defect associated with moderately raised tHcy is a point mutation C to T substitution at nucleotide 677 (that causes an alanine to valine substitution) in the coding region of the gene for MTHFR. The allele frequency of the MTHFR C 677 T mutation among Asian population is 35% with a homozygous rate of 12% which is similar to those in the North American Caucasian populations. Homozygosity for the C 677 T mutation is characterized by MTHFR thermolability, which decreases the specific activity of the enzyme by 50% (17, 18). Few studies in Japanese population have shown a positive association between MTHFR thermolability and cardiovascular events (19). A recent meta-analysis has concluded that although the C 677 T mutation is a major cause of mild hyper homocysteinemia, the mutation does not increase cardiovascular risk (20). Other genetic defects in enzymes that are associated with hyperhomocysteinemia include methionine synthase deficiency and disorders of vitamin B12 metabolism that impair methionine synthase activity.

Nutritional deficiencies in the vitamin cofactors (folate, vitamin B12, and vitamin B6) required for homocysteine metabolism may also cause hyperhomocysteinemia. Selhub *et al* (21) have suggested that inadequate plasma concentrations of one or more B-complex vitamins are contributing factors in approximately two thirds of all cases of hyperhomocysteinemia. Plasma homocysteine concentrations increase with elevations in creatinine and are typically elevated in chronic renal failure,

not because of impaired urinary excretion but because of impaired metabolism of homocysteine by the kidney, the major route by which homocysteine is cleared from plasma (22). Hyperhomocysteinemia has also been reported in patients with hypothyroidism, pernicious anemia, several types of carcinoma including breast, ovarian, and pancreatic cancer, and acute lymphoblastic leukemia (23). Several drugs and toxins also increase plasma homocysteine concentrations. Methotrexate depletes folate which is the co-substrate for methionine synthase and causes increase in plasma homocysteine concentration (24). Phenytoin also interferes with folate metabolism. Theophylline may cause hyperhomocysteinemia by antagonising the synthesis of pyridoxal phosphate (vitamin B6) (25). Cigarette smoking also interferes with the synthesis of pyridoxal phosphate (26).

In general, homocysteine levels tend to increase with age. Men have higher homocysteine levels than age matched women. Homocysteine levels tend to increase after menopause which explains the increased risk of CAD in older women (27).

PATHOPHYSIOLOGY

Evidence from several studies suggest that homocysteine obviously plays a causal role in atherothrombotic disease. Accumulation of homocysteine in the blood has a direct toxic effect on the endothelium by impairing EDRF –NO production as well as stimulating smooth muscle cell proliferation. It may interfere with the vasodilation and anti – coagulation functions of nitric oxide (NO) (28). Van den Berg *et al* (29) demonstrated impaired endothelial anticoagulant function in young patients with hyperhomocysteinemia and peripheral vascular disease. Although the exact mechanism of endothelial dysfunction is unknown, there is evidence that homocysteine exerts its effects by promoting oxidative damage.

Excess homocysteine can form homocysteine thiolactone, a highly reactive intermediate, which combines with low density lipoprotein to form aggregates that are taken up by intimal macrophages and incorporated into foam cells within nascent atheromatous plaques (30). The auto – oxidation of homocysteine also produces reactive oxygen species including superoxide and hydrogen peroxide which initiate lipid peroxidation and support the oxidation of low density lipoprotein (31).

McCully has suggested that homocysteine thiolactone impairs oxidative phosphorylation by facilitating the conversion of mitochondrial thioretinaco – ozonide to thioco, and promotes the proliferation and fibrosis of smooth muscles (30). This homocysteine induced disturbance in oxidative metabolism also leads to overproduction of oxidative radicals that induce intimal injury, activate elastase, and increase calcium deposition. Homocysteine is also a potent mitogen for vascular smooth muscle cells. Tsai *et al* (33) have proposed that homocysteine promotes atherogenesis specifically by inducing the proliferation of vascular smooth muscle cells. Figure – 1 shows the adverse vascular effects of homocysteine in promoting atherothrombosis.

CONCLUSION

There is considerable epidemiological evidence, which confirms the importance of plasma homocysteine as a powerful predictor of future risk of coronary heart disease and other complications of atherosclerosis. Treatment of hyperhomocysteinemia varies with the underlying cause. However, an inexpensive vitamin supplementation with folic acid, vitamin B12 and vitamin B 6 is generally effective in reducing homocysteine concentrations. Several randomised, controlled trials evaluating the effects of folic acid based supplements on homocysteine concentrations have been conducted over the last decade. In most patients, folic acid alone, and in combination of vitamin B12 and B6, has been shown to reduce homocysteine concentrations within four to six weeks after the initiation of therapy (34).

However, no study has yet demonstrated that lowering of homocysteine by vitamin supplementation decreases the cardiovascular morbidity or mortality. Avoidance of excessive meat intake and increased consumption of fresh vegetables and fruits is a dietary measure, which has many health benefits, including a potential to reduce elevated homocysteine levels. The other reasonable approach is to determine levels of fasting homocysteine in high risk patients and it may be advisable to increase their intake of vitamin fortified foods and / or to suggest the daily use of supplemental vitamins. Several large scale randomised trials like Heart Outcomes Prevention Evaluation (HOPE – 2) Study, McMaster University, Canada, Study of the Effectiveness of Additional

Reductions in Cholesterol and Homocysteine (SERCH), Clinical Trial Service Unit, Oxford, U.K, Cambridge Heart Antioxidant Study (CHAOS –2) University of Cambridge, U.K, Bergen Vitamin Study, University of Bergen Norway, Women's Antioxidant and Cardiovascular Disease Study (WACS) Harvard

Medical School, U.S.A, Prevention with a combined inhibitor and folate in Coronary Heart Disease (PACIFIC) study, University of Sydney, Australia, and many others are ongoing to assess the effect of homocysteine – lowering by vitamin supplementation on risk of vascular disease.

TABLE 1.

Causes of Hyperhomocysteinemia:

Primary:

Inherited enzyme defects:
Cystathionine beta synthase
Methylene tetrahydrofolate reductase
Methionine synthase.

Secondary:

- a) Physiological:
 - Increasing age.
 - Male gender
 - Menopause
- b) Life style factors:
 - Tobacco use
 - Coffee consumption
- c) Vitamin Deficiencies:
 - Folic acid
 - Vitamin B₁₂
 - Vitamin B₆
- d) Systemic disorders:
 - Chronic Renal failure
 - Pernicious anemia
 - Hypothyroidism
 - Systemic lupus erythematosus
 - Psoriasis
- e) Malignancies:
 - Breast
 - Ovary
 - Pancreas
 - Acute lymphoblastic leukaemia.
- f) Drugs:
 - Dihydrofolate reductase inhibitors (Methotrexate)
 - Folic acid antagonist (Phenytoin, Carbamazepine)
 - Vitamin B₁₂ antagonist (Nitrous oxide)
 - Vitamin B₆ antagonist (Theophylline)
 - Cholesterol lowering drugs (Cholestyramine, Niacin)

FIGURE 1.
ADVERSE VASCULAR EFFECTS OF HOMOCYSTEINE.

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