Raised Plasma Homocysteine: An Emerging Risk Factor for Ischaemic Heart Disease

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Abstract

Only one half to two thirds of atherosclerotic vascular diseases can be explained by classical risk factors like smoking, diabetes mellitus, hypertension, dyslipidaemia, family history of premature atherosclerotic vascular diseases, physical inactivity, obesity etc. Some other variables appear to contribute to the development of atherosclerotic vascular diseases which include estrogen deficiency, lipoprotein (a), plasma fibrinogen, plasminogen-activator inhibitor type I, endogenous tissue plasminogen activator (tPA), C-reactive protein and homocysteine. Over the last several years, investigators undertook extensive research work, in home and abroad, to determine the contribution of plasma homocysteine in the pathogenesis of atherosclerotic vascular diseases. So far the research work indicates, raised plasma homocysteine appears to be a potential risk factor for ischaemic heart disease.

Introduction

Despite steady progress in treatment of cardiovascular diseases, people are still dying of these diseases, although at later ages. By the year 2020, Coronary heart disease (CHD) and stroke will hold first and fourth places respectively in the World Health Organization's list of leading causes of disability. A worldwide epidemic of cardiovascular diseases is evolving, and atherosclerosis, often with thrombosis superimposed, is by far the most frequent underlying cause.

In the past, when a patient was considered at risk for atherosclerotic heart disease (AHD), the healthcare provider began management by "rounding up the usual suspects": smoking, obesity, hypercholesterolemia, family history, physical inactivity, diabetes mellitus, hypertension and other co-morbidities. Yet, in recent years, it has been suggested that only one half to two thirds of risk for atherosclerotic vascular disease can be explained by classic risk factors. Other variables that have come under scrutiny for their potential contribution include estrogen deficiency,
lipoprotein (a), plasma fibrinogen, plasminogen-activator inhibitor type I, endogenous tissue plasminogen activator (tPA), C-reactive protein and homocysteine.

First suspicion on homocysteine as a risk factor

There is a growing recognition that high level of homocysteine is associated with heart disease. This started in the late 1960s when a pathologist in Boston encountered two children with homocysteinuria, who, despite being very young, had advanced atherosclerosis. The pathologist concerned, Kilmer McCully, was, however, given a hard time for putting forward the suggestion of a possible link between homocysteine and the formation of atheromatous plaque.

Biochemistry and metabolism

Homocysteine is a sulfur-containing amino acid derived from the metabolism of dietary methionine, an essential amino acid that is abundant in animal protein and produced in small amount in the human body. It is metabolized by transsulfuration (which depends on vitamin B6) and remethylation (which relies on folate and vitamin B12). Although the physiological role of homocysteine remains unclear, the methyl group that is released during its formation plays a key role in numerous body processes including the synthesis of hormones, proteins and DNA. Methionine is the major methyl group donor in mammals. The recommended dietary allowance for methionine in U.S. adults is 0.9 gm /day. The first step in the metabolism of methionine is the formation of intermediate S-adenosylmethionine, which is an important methyl donor in many transmethylation reactions. S-adenosylmethionine is demethylated to form S-adenosylhomocysteine, which is then hydrolysed to adenosine and homocysteine. Homocysteine is then metabolised by either transsulfuration or remethylation pathways:

(a) Trans-sulfuration: When methionine stores are adequate or in condition of high protein intake, approximately 50% of homocysteine enters the trans-sulfuration pathway, where it is irreversibly combined with serine by the vitamin B6 (pyridoxine) dependent enzyme cystathionine beta-synthase to form cystathionine which is then metabolised to cysteine. Cysteine may be metabolised further to sulphate and excreted in urine.

(b) Remethylation: Under condition of low protein intake or when methionine conservation is necessary, homocysteine is metabolised primarily via one of two methionine-conserving remethylation pathways. In one, homocysteine is reconverted to methionine by transfer of a methyl group from 5-methyltetrahydrofolate in a reaction catalysed by cobalamin (vitamin B12) dependent enzyme methionine synthase. The formation of 5′methyltetrahydrofolate (methyl donor) depends on the presence of 5, 10-methylene-tetrahydrofolate (derived from dietary folate) and the enzyme 5, 10-methylene tetrahydrofolate reductase (MTHFR), which requires vitamin B2 (riboflavin) as a co-factor. The other remethylation pathway operates independently of vitamin B12 and folate but uses betaine as a methyl donor and requires betaine-homocysteine methyltransferase (BHMT).

Circulating forms of Homocysteine

Homocysteine is present in plasma in four forms: about 1% circulates as the free thiol; 70-80% is bound to plasma proteins, chiefly albumin by disulfide linkage; 10-15% combines with itself to form the dimer homocysteine and the remainder 10-15% combines with other thiols including cysteine to form homocysteine-cysteine mixed disulfide. The term "total plasma (or serum) homocysteine" (tHcy) refers to the combined pool of all four forms of homocysteine.

Definition of Hyperhomocysteinaemia and Determination

Hyperhomocysteinaemia is usually defined by using arbitrary cut off points—for example, above the 95th percentile or more than two SDs above the mean of values obtained from fasting, healthy controls. Normal levels of fasting plasma homocysteine are considered to be between 5 and 15 µmol/L. Moderate, intermediate and severe hyperhomocysteinaemia refer to fasting
concentrations between 16 and 30, between 31 and 100, and >100 µmol/L, respectively \(^\text{11}\). Because variable changes in homocysteine levels have been observed postprandially, it is customary to obtain measurements in the fasting state. In some subjects, fasting levels may be in normal range, but a high plasma homocysteine level may be seen after a methionine loading test \(^\text{12}\). This test is performed by the ingestion of methionine (0.1 gm/kg body weight) after which plasma homocysteine levels are measured at varying intervals (2 to 8 hours) \(^\text{9}\). This test is limited by time and cost constraints and is of limited practical value in most circumstances. Moreover, standard reference ranges for postmethionine load values in many populations have not been clearly established \(^\text{13}\). Given the cost and time required to perform the methionine loading study, a single fasting sample is accepted as the most cost effective test \(^\text{9}\).

**Factors Influencing Homocysteine Metabolism**

1) Genetic defects in homocysteine metabolism:
   (a) Transsulfuration abnormalities: Diminished or absent cystathionine-beta-synthase activity (chromosome 21)
   (b) Remethylation abnormalities:
      i. Abnormal methyl tetrahydrofolate reductase (absent or thermolabile variant)
      ii. Abnormal methionine synthase

2) Age/gender
   (a) Homocysteine increases with age
   (b) Homocysteine levels: men >age matched women
   (c) Postmenopausal women: homocysteine levels increase

3) Nutrition
   (a) Vitamin B\(_6\) deficiency
   (b) Vitamin B\(_{12}\) deficiency
   (c) Folate deficiency

3) Disease states
   (a) Severe psoriasis associated with increased homocysteine level (possibly related to lower folate levels).
   (b) Malignancies: acute lymphoblastic leukaemia, carcinoma of the breast, ovary and pancreas, increase levels.
   (c) Connective tissue disorders (Rheumatoid arthritis and systemic lupus erythematosus) increases tHcy leve.
   (d) Hypothyroidism increases tHcy level.
   (e) Chronic renal failure, increases homocysteine, lowered with dialysis.

4) Medications
   (a) Increase homocysteine:
      i. Folate antagonists (methotrexate, phenytoin, carbamazepine)

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**Figure 1:** Metabolic pathways of homocysteine metabolism (after Mayer et al. 1996) \(^\text{9}\).
ii. Vitamin B₆ antagonists (theophylline, oestrogen containing oral contraceptives, niacin).

iii. Cholestyramine, colestipol and metformin (affect folate and cobalamin absorption)

iv. Cyclosporine (reduces renal function). Fibric acid derivatives (possibly alter renal function)

(b) Decrease homocysteine:
1. Penicillamine and N-acetylcysteine (affect plasma bioavailability by altered disulfide exchange)
2. Betaine (enhances remethylation)

Pathophysiologic Mechanisms of Vascular Disease:

Both clinical and experimental evidence suggests that the atherogenic propensity associated with hyperhomocysteinemia is caused by endothelial dysfunction and injury which in turn is followed by platelet activation and thrombus formation. Numerous mechanisms have been suggested by which hyperhomocysteinemia may contribute to atherothrombotic vascular disease which is as follows:

1. **Endothelial dysfunction:** Although the exact mechanism of endothelial dysfunction is unknown, evidence has been shown to be related primarily to-
   a) The generation of potent reactive oxygen species, including superoxide and \( \text{H}_2\text{O}_2 \) during auto-oxidation of homocysteine, both of which have been linked to damage of endothelial lining of arterial vessels.
   b) Impaired production of endothelium derived nitric oxide and endothelial dysfunction, as evidenced by impaired endothelial dependent vascular reactivity in primates and in humans with elevated homocysteine levels.

2. **Vascular growth:** Homocysteine (Hcy) causes stimulation of growth promoting signal transduction pathway, e.g. the mitogen activated protein kinase signal transduction pathway in vascular smooth muscle cells, promoting mitogenesis. Hcy also enhances DNA synthesis in human vascular smooth muscle cells and enhances human vascular smooth muscle cell proliferation and collagen expression.

3. **Lipid peroxidation:** Auto-oxidation of Hcy produces other cytotoxic reactive oxygen species, including the superoxide anion radical and hydroxyl radical. Superoxide dependent formation of the hydroxyl radical has been shown to initiate lipid peroxidation, an effect that occurs at the level of the endothelial plasma membrane and within lipoprotein particles. Hcy also causes oxidation of LDL through generation of superoxide anion radical and may, therefore, promote cellular uptake of modified LDL, an important step in atherosclerotic process.

4. **Effect on platelet and clotting factors:** Elevated levels of Hcy may interfere with the anti-thrombotic and fibrinolytic mechanisms of the endothelium, making it more prothrombotic. Hcy thiolactone causes platelets to aggregate - a part of clotting cascade. Hcy alters the normal antithrombotic phenotype of endothelium by enhancing the activities of factor XII, X and factor V and depressing the activation of protein C. Hcy also inhibits the expression of thrombomodulin, induces the expression of tissue factor and suppresses the expression of heparan sulfate by endothelium. All of these effects ultimately facilitate the formation of thrombin and create a prothrombotic environment. Hcy decreases tissue plasminogen activator activity & inhibit fibrinolysis. Recent data shows that Hcy also directly blocks the tissue plasminogen activator binding domain of annexin II. This would be expected to inhibit thrombolysis and thereby promote thrombosis.

A potentially unifying hypothesis of the vascular damage associated with hyperhomocysteinemia relates to the formation of oxygen free radicals, which cause oxidative vascular damage, proliferation of smooth muscle cells, alteration in endothelial function and structure and increased thrombogenicity that ultimately leads to atherothrombosis.
E) Epidemiologic associations between hyperhomocysteinaemia and cardiovascular risk:

Atherothrombotic vascular events were linked to raised total homocysteine in patients with homocystinuria in 1969 but it was not until 1976 that a controlled study showed a clear association between moderately raised total homocysteine and atherosclerotic disease. Since then a number of studies have investigated the relationship of homocysteine and risk of vascular diseases, including coronary artery, cerebrovascular and peripheral vascular disease as well as deep venous thrombosis. The results of some of the important studies are reviewed as follows:

**Cross sectional and Retrospective case control studies:** Many cross-sectional and retrospective observational studies have examined the association between plasma Hcy level and cardiovascular risk and most support the existence of such an association. Boushey et al., reported a meta-analysis of 27 observational studies (23 cross-sectional or retrospective case-control and four nested case-control studies based on prospective cohorts), including about 4000 patients. A raised t-Hcy (usually defined as above the 90th or 95th percentile of controls) was associated with an increased risk of fatal and nonfatal atherosclerotic vascular disease in the coronary (Odds ratio (OR) 1.7; 95% Confidence interval (CI) 1.5-1.9), cerebral (OR 2.5, CI, 2.0-3.0), and peripheral (OR 6.8, CI, 2.9-15.8) circulations. The magnitude of risk was similar to that for other risk factors, such as hypercholesterolaemia and smoking, and it was estimated that about 10% of coronary heart disease in the general population might be attributable to homocysteine. Boushey et al.(1995) also estimated, from an analysis that assumed a graded, linear relation between homocysteine levels and vascular risk, that a 5 µmol/L increase in tHcy was associated with an increase in vascular risk of about one-third, which is of similar magnitude to an increase in plasma cholesterol of 19 mg/dl.

**Table 1. Case-Control studies of Hcy and Cardiovascular risk**

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Patient selection</th>
<th>Age (y)</th>
<th>Sex</th>
<th>Participants (Case/Control)</th>
<th>Total Hcy</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Graham et al. (1997) Europe</td>
<td>Case-Control</td>
<td>Men and women with vascular disease</td>
<td>47.2±0.3</td>
<td>M/F</td>
<td>750/800</td>
<td>11.3</td>
<td>RR=2.2 (1.6-2.9)</td>
</tr>
<tr>
<td>Hopkins et al., (1995) USA</td>
<td>Case-Control</td>
<td>Men and women with CAD and F/H of IHD</td>
<td>62.0±11.0</td>
<td>M/F</td>
<td>304/231</td>
<td>M-13.7</td>
<td>M:OR=13.8 (3.5-55)</td>
</tr>
<tr>
<td>Dalery et al., (1995) Canada</td>
<td>Case-Control</td>
<td>Men and women with angiographic CAD</td>
<td>25-64</td>
<td>M/F</td>
<td>420/521</td>
<td>M-11.7</td>
<td>M:p&lt;0.001</td>
</tr>
<tr>
<td>Robinson et al., (1995) USA</td>
<td>Case-Control</td>
<td>Men and women with angiographic CAD</td>
<td>38-68</td>
<td>M/F</td>
<td>162/155</td>
<td>M-13.9</td>
<td>M:OR=0.024 (1.7-4.7)</td>
</tr>
<tr>
<td>Malinow et al., (1996) France and Ireland</td>
<td>Case-Control</td>
<td>Men with previous MI</td>
<td>20-59</td>
<td>M</td>
<td>150/584</td>
<td>Ire-15.5</td>
<td>Ire-OR 3.42 (1.6-7.2)</td>
</tr>
</tbody>
</table>
Since publication of this meta-analysis, many additional observational studies have been done. Most of these studies have also reported an association between hyperhomocysteinemia and atherosclerotic vascular disease. The recent large European Concerted Action Project which involved 750 patients with arterial vascular disease and 800 controls, confirmed that an elevated plasma homocysteine level was an independent risk factor for cardiovascular disease (OR 2.2, CI, 1.6 to 2.9) and calculated that an increase of 5 µmol/L in fasting basal Hcy level was associated with a relative risk for cardiovascular disease of 1.35 (CI 1.1, to 1.6) in men and 1.42 (CI, 0.99 to 2.55) in women.

Conclusion

References


31. Kabir S, Association of plasma total homocysteine level with the number of major coronary arteries severely narrowed in patients with ischaemic heart diseases. MD theses 2003; National Institute of Cardiovascular Diseases, Dhaka, Bangladesh.

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