



HYPERHOMOCYSTEINEMIA AND ATHEROSCLEROSIS – AN OVERVIEW

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ABSTRACT

Indians are reported to have a very high prevalence of premature Coronary artery disease (CAD). A link between plasma homocysteine concentrations with vascular disease was first suggested by the observation made in patients with homocystinuria having arteriosclerosis. The recent interest in this topic has been generated by the evidence, that people with hyperhomocysteinemia have an increased risk of cardiovascular disease (CAD). On the basis of retrospective and epidemiological studies it is now widely accepted that hyperhomocysteinemia as an independent risk factor for cardiovascular disease. Present article gives an overview of the structural features, physiological significance and clinical implications of homocysteine with vascular disease.

KEY WORDS

Atherosclerosis, Coronary artery disease, Homocysteine, Hyperhomocytseinemia.

INTRODUCTION

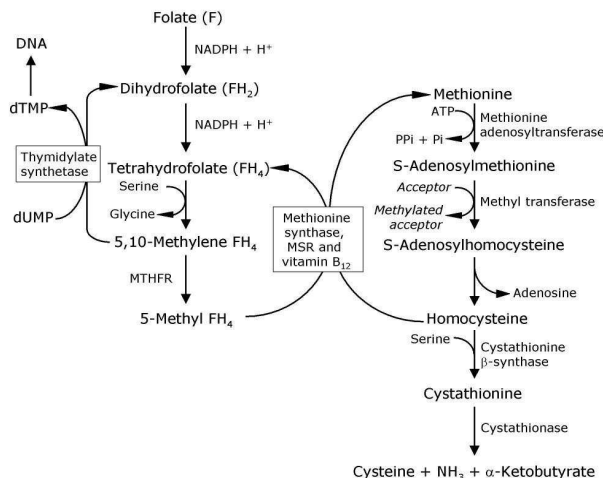
Homocysteine is a sulphur containing amino acid derived primarily from the break down of dietary methionine. In 1930 DuVigneaud (1) and Butz discovered these compounds, which are homologous to the sulphur containing amino acid cysteine and cystine. The compounds, which are homologous to cysteine, are called homocysteine the reduced (sulfhydryl) form and the one homologous to cystine are called homocystine the oxidized (disulphide) form. Homocysteine is present only in trace amounts in the diet and dietary homocysteine does not under normal circumstances appear to affect the plasma homocysteine level.

HOMOCYSTEINE METABOLISM

Homocysteine lies at a branch point in one carbon metabolism between two metabolic pathways (remethylation and trans-sulphuration) in all cells. Figure-1 shows the metabolic pathway of homocysteine. In the remethylation

pathway, homocysteine accepts a methyl group from the methyl-tetra hydro folate to form methionine. The remethylation reaction catalyzed by methionine synthase requires vitamin B₁₂ as a cofactor. Enzyme methyl tetra hydro folate reductase (MTHFR) plays an important role by supplying methyl groups as methyl tetra hydro folate for homocysteine remethylation. An alternative remethylation pathway utilizing betaine as methyl donor is confined only to liver. This reaction requires the enzyme betaine homocysteine methyl transferase. Much of the methionine is activated to form S-adenosyl methionine (SAM), which is the chief donor of methyl groups for methyl transferases involved in the synthesis of DNA, proteins and phospholipids. The loss of methyl group from SAM results in the production of S-adenosyl homocysteine, which in turn hydrolyzed to form homocysteine.

Figure-1
Homocysteine metabolism





In the trans-sulphuration pathway, homocysteine condenses with serine to form cystathionine in an irreversible reaction catalyzed by vitamin B₆ dependent cystathionine β synthase. Cystathionine is subsequently hydrolyzed to form cysteine, which may in turn be incorporated into glutathione or further metabolized to sulfate and excreted in the urine (2).

Under normal circumstances the flow of methionine to cystathionine accounts for half of all methionine metabolisms. The remaining half of total body homocysteine is remethylated by methyl groups derived in equal amounts from methyltetrahydrofolate or betaine.

The intracellular levels of homocysteine are highly regulated, and any increased production will be exported from the cells. Thus blood levels of homocysteine reflect intracellular concentrations of homocysteine and the homeostatic balance of the enzymes involved in methionine metabolism to ensure a supply of methyl groups for essential reactions in all cells (3).

Metabolic Intermediates of Homocysteine:

Homocysteine plays an important role in methionine (sulfur and methyl group) metabolism. Any alter in homocysteine metabolism will have a negative impact on the biosynthesis of key nutrients like S-adenosyl methionine (SAM), carnitine, chondratin sulfates, glutathione, epinephrine, glucosamine sulfate, coenzyme A, pantethine taurine. Homocysteine can be remethylated to form methionine and it can be degraded into cysteine. Once cysteine is generated it can be directed into several different pathways including synthesis of glutathione, acetyl CoA, Phospadenosinephosphosulfate and taurine.

Thus, homocysteine an intermediate product of methionine metabolism is located at a critical metabolic crossroad and therefore both directly and indirectly impacts all methyl sulfur group

metabolism occurring in the body. Experiments have demonstrated that high levels of homocysteine and adenosine accumulation in the cell, leading to inhibition of all methylation reactions (4).

Homocysteine occurrence:

Plasma contains both reduced and oxidized species of homocysteine. The reduced or sulfahydryl form is called homocysteine and the disulfide or oxidized form is called homocysteine. Disulfide forms also exist with cystines and proteins containing reactive cysteine residues (protein bound homocysteine) the latter oxidized forms are referred as mixed disulfides. The oxidized forms of homocysteine usually comprise 98–99% of total plasma homocysteine in human plasma, 80-90% of which is disulfide bound to plasma proteins, mainly albumin, 10-20% combines with itself to form the dimmer homocysteine or with other thiols, including cysteine. The remaining 1% circulates as the free thiol. The term “total plasma homocysteine” (t Hcy) refers to all the four forms of homocysteine (5).

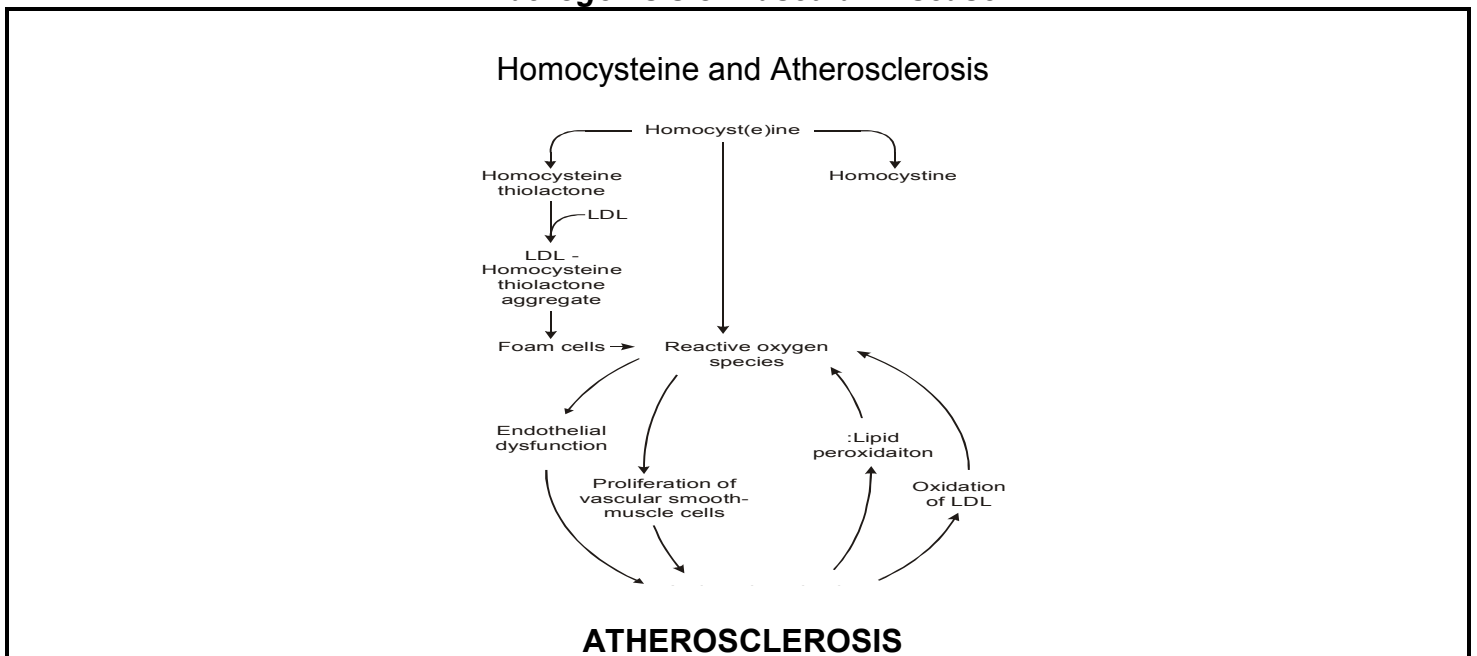
Determinants of Hyperhomocysteinemia:

A number of enzymes essential cofactors and the availability of the important co substrate methyltetrahydrofolate regulate plasma homocysteine concentrations. Hence the causes of hyperhomocysteinemia are multifactorial. Hyperhomocysteinemia can be caused by either physiological or pathological factors (6). Physiological factors include age gender whereas the pathological factors may be either genetic or acquired causes (7). Genetic causes include the deficiency of metabolic enzymes of homocysteine metabolism. Acquired type of hyperhomocysteinemia is due to nutritional, life style, state of health and medications (8). Thus, determinants of total plasma homocysteine are complex and involve demographic, genetic and acquired factors. Table-1 shows the different causes of hyperhomocysteinemia.

Table-1
Factors Influencing Homocysteine levels

- I. Genetics
 - A. Trans-sulfuration abnormalities: diminished or absence of cystathionine β -synthase activity.
 - B. Remethylation abnormalities: Abnormal Metylenetetrahydrofolate reductase (MTHFR) (absent or thermo labile variant), abnormal methionine synthase.
- II. Age / Gender
 - A. Increases with age
 - B. Homocysteine levels in men more than age matched women
- III. Nutrition
 - A. Vitamin B₆ deficiency
 - B. Vitamin B₁₂ deficiency
 - C. Folate deficiency
- IV. Disease States
 - A. Chronic renal failure
 - B. Cancer, acute lymphoblastic leukemia
- V. Medications
 - A. Increase homocysteine
 1. Methotrexate, depletes 5- methyl tetra hydro folate
 2. Azaribine, Vitamin B₆ antagonist
 3. Nitrous oxide, inactivates vitamin B₁₂
 4. Phenytoin, interferes with folate metabolism
 5. Carbamazepine, interferes with folate metabolism
 - B. Decrease homocysteine
 - Penicillamine, metabolically stable cysteine analogue

Figure – 2
Pathogenesis of Vascular Disease





It is hypothesized that reduced homocysteine directly alters vascular cell function. Figure-2 shows the adverse vascular effects of homocysteine in promoting atherosclerosis. Because reduced homocysteine undergoes oxidation in vivo, one could argue that the homocysteine oxidation products such as hydrogen peroxide, super oxide anion radical and other reactive oxygen species are the injurious agents. Thus homocysteine is acting through its oxidation and formation of reactive oxygen species to the vascular endothelium. Numerous studies have shown the influence of homocysteine on vascular pathology by effecting the endothelial surface, vascular smooth muscle cells, connective tissue, and interactions with plasma lipoproteins, clotting factors and platelets (9). The endothelium has received considerable attention as the final common pathway of homocysteine induced vascular injury (10).

Homocysteine indirectly regulates vascular tone and permeability in which nitric oxide plays an important role. Endothelium has fibrinolytic functions that are mediated through tissue plasminogen activator (tPA) and plasminogen activator inhibitor-1. The endothelium may also influence composition of the sub-endothelial matrix and smooth muscle cell proliferation. Many of these normal properties may become damaged in presence of elevated homocysteine levels.

High concentrations of homocysteine impair generation of NO (11) and also increase, cytotoxic reactive oxygen species including super oxide anion radical, hydroxyl radical and hydrogen peroxide. Harker et al (12) and wall et al (13) suggested that endothelial damage is mediated by hydrogen peroxide a by-product of homocysteine auto oxidation. Homocysteine has pro-oxidative effects forming hydrogen peroxide in presence of copper or ceruloplasmin (14).

Thus hyperhomocysteinemia causes oxidative stress, which results in oxidation of LDL through the superoxide anion radical. Further homocysteine thiolactone, a byproduct of

homocysteine auto-oxidation combines with native LDL to form oxidized or modified LDL. This oxidized LDL is then taken up by macrophages to form foam cells, which develops into atherosclerosis. Huges et al (15) have reported that vascular cytotoxicity of oxidized LDL has been linked to its lipid peroxidation effect.

Homocysteine also increases the formation of highly atherogenic oxysterol (16). Homocysteine interact with nitric oxide to form S-nitroso thiols or S-nitroso homocysteine (17), and when homocysteine concentrations were elevated the availability of nitric oxide depletes which results in endothelial dysfunction. Homocysteine induced disturbance in oxidative metabolism also leads to overproduction of oxidative radicals and subsequently induce intimal injury, activate catalase and increase calcium deposition. Homocysteine contributes to the deposition of sulfated glycosaminoglycans in the matrix because of increased homocysteine thiolactone production (18). These sulfated glycosaminoglycans affect the collagen synthesis and leads to proliferation.

Thus, in general homocysteine facilitates the generation of hydrogen peroxide. By creating oxidative damage to LDL-cholesterol and endothelial cell membrane, hydrogen peroxide can then catalyze injury to vascular endothelium. Because of the role of sulfate compounds in the formation of amino sugars, which are also needed to form the basement membrane of blood vessels, high levels of homocysteine may contribute to the formation of blood vessels that are more susceptible to oxidative stress (19). The combination of oxidative damage and endothelial collagen instability results in the formation of atherosclerotic plaques

Also remethylation of homocysteine and subsequent formation of S-adenosyl methionine (SAM) is critical for biosynthesis of L-carnitine, coenzyme Q and creatine. Similarly the transsulfuration pathway must be functioning properly for the optimal biosynthesis of cysteine,



glutathione, pantothenic acid and taurine. All of these nutrients have been used clinically to either reduce oxidative stress, or to improve risk factor markers of heart diseases.

The association between raised homocysteine and thrombosis was reported by McCully (20), who demonstrated thrombovascular abnormality in homocysteinuria patients. In the last three decades several studies (21,22) showed an association between homocysteine and CAD. Several recent studies investigated the contribution of homocysteine to CAD risk both among immigrant Indians (23, 24) and those living in India (25, 26). Boushy et al (27) showed homocysteine as an independent graded risk

predictor for atherosclerotic disease in coronary and cerebral and peripheral vessels. Our study (28) showed that homocysteine is the best predictor of CAD amongst other conventional CAD risk factors, and also other studies from south India (29, 30) showing the association of homocysteine with CAD risk. Though there are many studies related to homocysteine and CAD, things like the cut off value for homocysteine, consequence of hyperhomocysteinemia on diabetes, hypertension, and hyperlipidemia and timing of homocysteine measurements has to be ascertained. Further studies in this regard would be useful in the management of hyperhomocysteinemia.

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