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Homocysteine as an independent and dependent causative factor of Cardio Vascular Diseases

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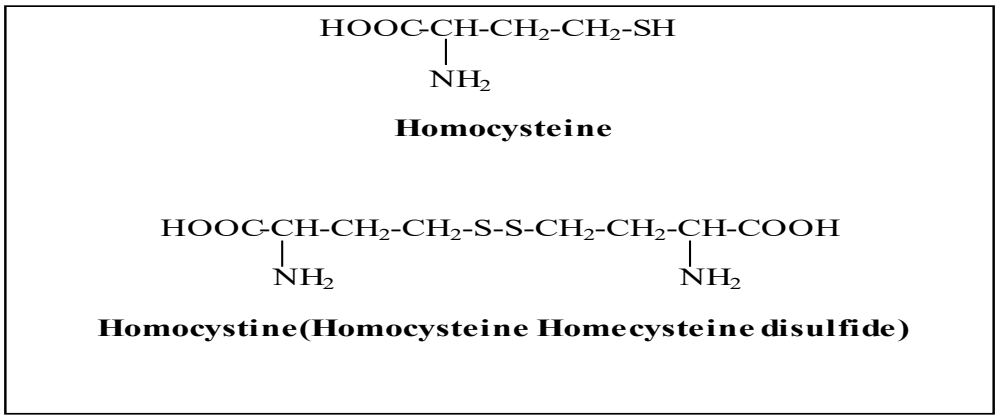
ABSTRACT

Homocysteine/homocystine (Hcy), an endogenous sulfur containing non proteinous amino acid synthesized during the catabolism of essential dietary amino acid methionine. Once homocysteine is produced, it is metabolized in the body through one of two possible pathways - remethylation or transsulfuration. Plasma homocysteine concentrations may differ, depending on which metabolic homocysteine pathway is preceded in the body. Genetics, nutritional status, and life style factors are the major determinants of plasma Hcy levels. Even a mild impairment in remethylation or transsulfuration pathways may lead to increase in plasma fasting Hcy concentrations (≥15µmol/litre). The biological relevance of Hcy metabolism and its association with various factors makes it an important etiological factor for cardio vascular diseases. Elevated levels of Hcy induces endothelial damage, produces vascular inflammation, decreases bioavailability of nitric oxide (NO), increases oxidative stress and have a cytotoxic effect by modulating vascular cell function. Thus, Hcy act as independent biomarker of cardiovascular diseases and it can also promote other risk factors such as hypertension and diabetes. Intake of B-complex vitamins through diet or supplements may be considered the best therapeutic options for management of elevated Hcy level in body.

Key words: Homocysteine (Hcy), Cardio Vascular Disease, B-complex vitamins

INTRODUCTION: The sulfur containing amino acids, methionine and cysteine have specific roles in cell metabolism. For instance, methionine serves as a substrate for S-adenosylmethionine, which is vital for methylation of nucleic acids, proteins, and lipids. Cysteine/cystine is a substrate for glutathione, an important intracellular antioxidant and H_2S, a gas that can induce endothelial-dependent relaxation. Cysteine is a non-essential amino acid because it is synthesized from the essential amino acid methionine in body. However, the connective link between methionine and cysteine conversion is homocysteine. Homocysteine/ homocysteine (Hcy) is an endogenous sulfur containing non proteinous amino acid that occupies a central location in the metabolic pathways

of thiol compounds. The word endogenous clarifies that homocysteine cannot be supplemented through diet. It is an intermediate formed during the catabolism of the essential dietary amino acid methionine (Castro *et al* 2006). The total plasma/serum homocysteine (tHcy) reflects the combined pool of free (30%), bound (65%), and reduced or oxidized forms (1.5-4%) of Hcy in the circulatory system (Klee *et al*, 2000).



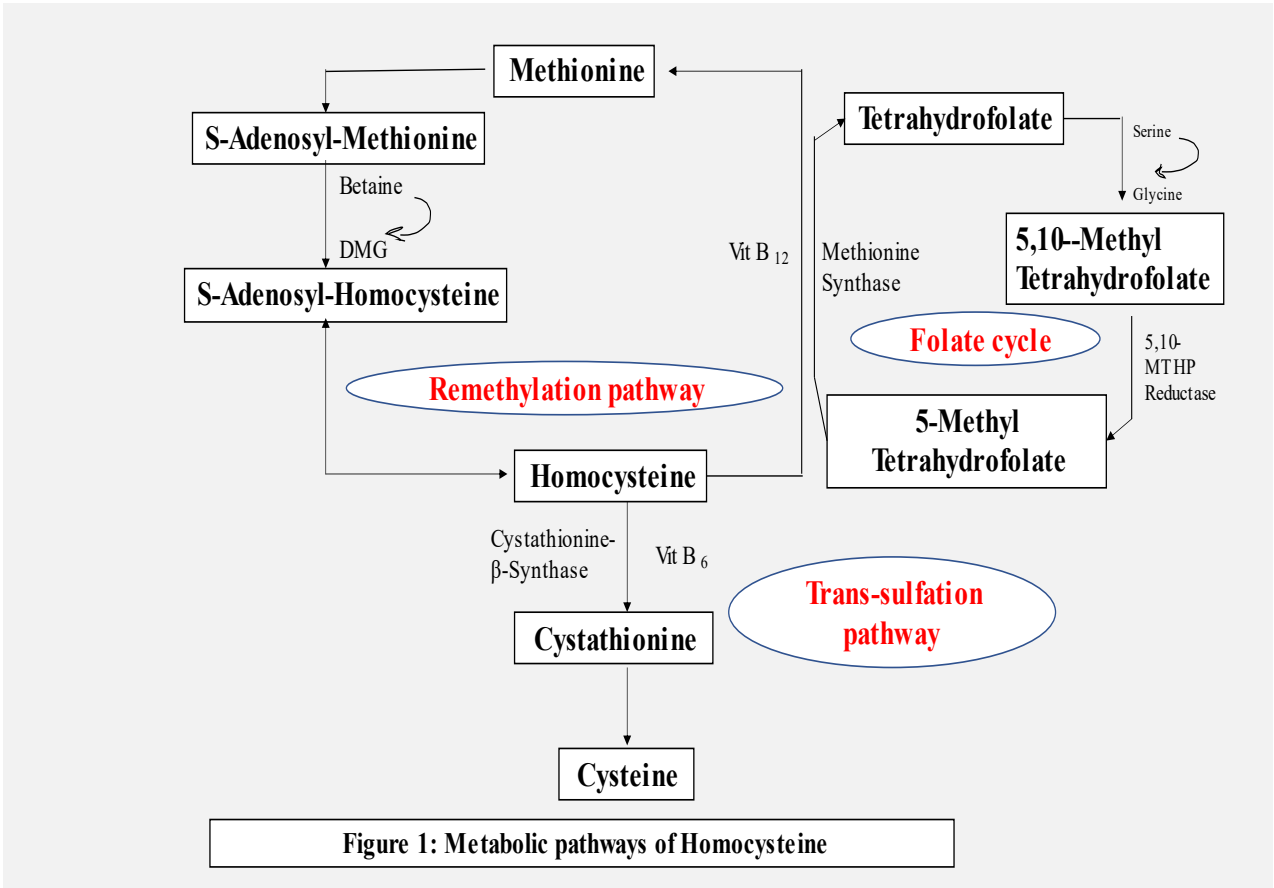


Figure 1: Metabolic pathways of Homocysteine

Source: Guilliams., 2004

HOMOCYSTEINE METABOLISM: Hcy is an intermediate of methionine metabolism, with the methionine derived primarily from dietary protein. This pathway involves the formation of S-adenosylmethionine (SAM) from methionine, which subsequently transfers a methyl group to any methyl acceptor molecules like DNA, proteins and neurotransmitters and forms S-adenosylhomocysteine (SAH), which is subsequently converted to Hcy. Homocysteine is then either converted back to methionine by remethylation or further metabolized to cysteine via the trans-sulfuration pathway. Remethylation primarily occurs when a methyl group is transferred from methyltetrahydrofolate (MTHF), the active form of the folic acid, by a methyltransferase enzyme requiring cobalamin (vitamin B_{12}) as a necessary cofactor. A secondary remethylation pathway, active primarily in liver and kidney cells, uses trimethyl glycine (betaine) as the methyl donor. The trans-sulfuration pathway requires two enzymatic reactions, both of which require the cofactor pyridoxal-5-phosphate, the active form of vitamin B_6 (Guilliams, 2004). The considered normal blood level of Hcy is <6.3 µmol/litre. The blood Hcy levels are high in males as compared to females in similar age groups. With increase of age, Hcy levels rises in both sex due to aging. varies in both sex groups.

Table 1: Total blood Hcy level (Anonymous, 2004)

Sex	Age	Hcy level (µmol/litre)	Therapeutic target (µmol/litre)	Desired (µmol/litre)
Male	12–59 years	4.3-9.9	> 10.4	<6.3
	>60 years	5.9-15.3		
Female	12–59 years	3.3-7.2		
	>60 years	4.9- 11.6		

FACTORS AFFECTING Hcy LEVELS: Plasma Hcy level is maintained by a complex interaction of acquired and genetic factors. Acquired factors includes nutritional status, age, sex, drugs, life style and pregnancy.

1. **Genetic factors:** Till 135 genes were identified in human body that either modulate the level of Hcy or modulated by elevated Hcy level. These genes are mainly involved in regulation of lipid and methionine metabolism, transport of lipids, apoptosis and cell signalling**.** A defect in these genes, primarily due to single nucleotide polymorphism (SNP) lead to an elevated Hcy level (Sharma *et al*, 2006). Increased Hcy can modulate the expression of certain genes that may either directly or indirectly lead to genetic disorders such as homocystinuria and polymorphism in MTHFR gene.

A. Homocystinuria: Homocystinuria was first identified in cases of rare inborn errors of metabolism characterized by the deficiency of Cystathionine-β-synthase enzyme. The homozygous form is very rare and associated with high level of fasting plasma Hcy level i.e.,>200µmol/L (Kumar *et al*, 2012). Clinical manifestations include mental retardation, thromboembolism, seizures, premature atherosclerosis and skeletal deformities. The dietary management of homocystinuria includes inclusion of nutritional supplements of cysteine, Vitamin B_6, B_{12}, B_9 and betaine from 50-200mg/day should be given with the low intake of methionine (Dwivedi *et al*, 2011).

B. Polymorphism in MTHFR gene: N_5, N_{10}-methylenetetrahydrofolate reductase (MTHFR) enzyme is an important enzyme of folate cycle. Homozygous deficiency of MTHFR is rare and occurs due to transition in MTHFR Gene at 677 codons with substitution of valine from alanine. Patients homozygous for the C677T mutation have slight elevations in Hcy level and are at the increased risk for premature vascular disease. Patients with the MTHFR genotype have higher folate requirements than individuals with a normal genotype. So, the dietary supplementation of folate (250-500µg/day) is sufficient for the management of this condition (Kumar *et al*, 2012).

2. Nutrition

A. B-Complex Vitamins: B-complex vitamins are important coenzymes in the Hcy metabolism. In remethylation pathway, vitamin B_{12}, folic acid and betaine are required. Vitamin B_6 is a coenzyme of cystathionin-β-synthase enzyme in transsulfuran pathway (Brosnan *et al*, 2004). Vitamin B_2 (Riboflavin) is a cofactor for methionine synthase. It is required in the folate cycle for regeneration of 5-methyltetrahydrofolate and the MTHFR enzyme, which requires FAD (Flavin Adenosine Dinucleotide) as a prosthetic group. In addition, riboflavin in another active form FMN (Flavin Mono Nucleotide) is required for the generation of pyridoxal phosphate which serves as a cofactor of cystathionine-β-synthase and other enzymes of the trans-sulfuration pathway. In deficiency of vitamin B_6, Hcy cannot be converted into cysteine leading to reduced cysteine availability for the various biological processes which are critical for tissue repair and synthesis. Methionine synthese enzyme requires vitamin B_{12} as a coenzyme in remethylation pathway. The primary biochemical function of folic acid is to transfer a single carbon in the synthesis of several metabolites in the body. Folic acid and B_{12} deficiency were found to be associated with elevated blood homocysteine level, which in turn was reported to be a major etiological risk factor of cardiovascular disease (Sukla and Ramon, 2012).

B. Other Nutrients: In Hcy metabolism, Betaine-homocysteine-S-methyltransferase (BHMT) is an enzyme that converts Hcy to methionine using betaine as a methyl donor. Thus, betaine can be used as a therapy for hyperhomocysteinamia patients with genetic defects, who are unresponsive for the pyridoxine, folic acid, and vitamin B_{12}. It also lowers plasma Hcy in healthy humans who have Hcy concentration in the normal range (Olthof *et al*, 2005). Supplementation of fish oil which is rich in ω-3 polyunsaturated fatty acids (PUFAs) may also reduce elevated Hcy levels (Pooya, 2010). Taurine an amino acid like substance can block methionine absorption from the diet,

thereby reducing available substrate for homocysteine synthesis (Ahn, 2009). Within the body, taurine is also synthesized within the pancreas through a pathway in which cysteine is oxidized to create cysteine sulphuric acid.

Complex carbohydrates, protein, fat and water-soluble vitamins are found to lower the Hcy levels by increasing Hcy metabolism into methionine and cysteine. While sugars and saturated fats are linked with elevated levels of Hcy in blood. Processed meat consumption is mainly associated with high Hcy levels because it contains more methionine and less B-complex vitamins (Konstantinova *et al,* 2007). During the food processing, most of the B-complex vitamins are lost due to heat and leaching in water. Vitamin C, vitamin-A and simple carbohydrates remained significantly associated with tHcy after adjustment for dietary B-complex vitamins, but not after additional adjustment of plasma folate and vitamin B_{12}.

3. Drugs: Drugs have both positive and negative effects on Hcy levels of blood. The important drugs which have potential to alter Hcy levels in blood. Lipid lowering drugs like fibrates and its derivative may increase Hcy level by 4µmol/L in 40-months period. Niacin supplementation (0.3mg/day) can elevate Hcy level up to 9µmol/L. As high doses of niacin decrease plasma vitamin B_6 level and subsequently increases Hcy concentrations. In antihypertensive drugs, diuretics and ACE inhibitors may enhance Hcy level by concomitant deterioration of renal function. However, Hcy levels are lower in patients who were taking β-blockers. Metformin a common antidiabetic drug (550-2550mg/day at least for 6 months) was also found to increase blood Hcy level by 0.8µmol/litre. B-complex vitamins supplementation decreases blood Hcy level by increasing Hcy metabolism. Supplementation of 10mg riboflavin, 15µg vitamin B_{12}, 1500µg folic acid and 100mg betaine may maintain Hcy levels within range (Dierkes *et al*, 2007).

4. Age and sex: Prior to puberty, both sex have optimum healthy levels of Hcy (about <6 µmol/L). During puberty, Hcy levels rises more in males than females, reaching up to 10µmol/L in men and 8µmol/L in women. Female sex hormones, estrogen and progesterone have negative effect on Hcy levels. Male sex is associated with higher Hcy level at all ages except in the old age females due to their reduced secretion of female sex hormones. Hcy levels also increases in the old age due to aging effects such as less enzyme secretion and reduced absorption of nutrients (Ganji and Kafai, 2006).

5. Life style factors: Sedentary life style, smoking and excess coffee consumption (>4 cups/day) is linked with elevated level of Hcy (Bazzano *et al,* 2003; Verhoef *et al,* 2002). Moderate alcohol consumption may raise Hcy levels (Bleich *et al,* 2001). Wine consumption may increase tHcy concentrations, whereas beer consumption seems to have no effect or even an inverse effect on t Hcy (Mennen *et al,* 2003).

6. Pregnancy: Hcy levels changes drastically in all the three trimesters of pregnancy. Total serum Hcy was found to be increased in the first trimester due to high folate utilization for neural tube closure of embryo. It is at the lowest level in second trimester due to high protein requirement which promotes remethylation process. In the third trimester it again becomes maximal (about

50% compared with non-pregnant women). A return to normal concentrations of Hcy is seen within 2-4 days postpartum. Folic acid supplementation was significantly effective to reduce tHcy level in pregnancy particularly in the last trimester (Holmes *et al*, 2005).

7. Renal dysfunction: Only free (unbound) Hcy is filtered and metabolized by the normal kidney filtration. Kidney stimulates conversion of bound Hcy to free Hcy by separating protein. In renal diseases, blood Hcy level increases due to improper glomerular filtration. So, the elevated levels of Hcy are commonly seen in renal patients, sometimes 3-4 times higher than normal individuals (Guilliams, 2004).

Thus, all these genetic and acquired factors have a complicated impact on Hcy metabolism. Most of the diseases like diabetes, kidney diseases and genetic congenital diseases are also important etiological factors for elevated Hcy level.

HYPERHOMOCYSTEINEMIA

Individuals with deficiency of B-complex vitamins or congenital metabolic defects are often incapable of metabolizing cellular Hcy to cysteine and methionine levels. Consequently, intracellular Hcy levels rapidly increase until the cell is forced to excrete excess Hcy into the bloodstream resulting in elevated serum Hcy levels. This condition is known as hyperhomocysteinemia. Increased levels of homocysteine are classified as (Kaul *et al*, 2006)

Moderate:	15-30µmol/litre
Intermediate:	30 to 100µmol/litre
High:	≥100µmol/litre

ASSOCIATION OF Hcy WITH CARDIOVASCULAR DISEASES (CVD)

The homocysteine hypothesis of arteriosclerosis was first proposed by McCully in 1969, when he observed autopsy on premature atherothrombosis of the peripheral, coronary and cerebral vasculature in children with homocystinuria. Hcy was found to be an independent biomarker of CVD which can also promote other causative factors of CVD. High Hcy in normal persons aged between 29-89 years were found only in 24.4% cases, while in atherosclerosis, 70.9% cases were found to have elevated Hcy levels (Rahman *et al*, 2006).

Hcy as an independent factor of CVD

1. Hcy induces endothelial cell damage, oxidizes low-density lipoproteins, and stimulates smooth muscle cell proliferation. Through these effects, increased Hcy were found to have positive association with development of atherosclerosis (Ghaedi *et al*, 2007).
2. In addition, high concentration of Hcy enhances thrombogenesis by increasing the activities of factor XII and factor V, depressing the activation of protein C, inhibiting the expression of thrombomodulin and suppressing the expression of heparan sulfate by endothelium. Association of Hcy with both pathological processes (atherosclerosis and thrombosis) makes it a potential risk factor for CAD (Iqbal *et al*, 2006; Narang *et al*, 2009).
3. Hyperhomocysteinemia causes desquamation of endothelium and impairs its regeneration (Poddar, 2001). Hcy also induce the expression and secretion of chemokines in vascular

endothelial cells (such as (MCP1) and interlukin 8 (IL-8). These cytokines attract monocytes and neutrophils at the sites of vascular injury and transform into macrophages which engulf oxidized LDL, and become foam cells. Foam cells are a source of reactive oxygen species which can play a role in other sequences of events that promote atherosclerosis.

4. Hyperhomocysteinemia is associated with the production of reactive oxygen species (ROS) in endothelial and smooth muscle cells. The mechanism of this oxidative stress is either on auto-oxidation of the highly reactive thiol group of Hcy or the formation of intracellular superoxide and peroxyl radicals with concomitant inhibition of cellular antioxidant enzymes, such as superoxide dismutase and glutathione peroxidase. Less nitric oxide (NO) availability, and increased activity of thiol group in hyperhomocysteinemia are the major sources of free radical generation (oxidative stress) which consequently increases the risk of cancer, hypertension and CVD (Forges *et al*, 2007).
5. In the presence of Hcy, fibroblasts produce excessively sulphated proteoglycans. So, the prepared connective tissue is granular rather than fibrillar due to excessively sulphated proteoglycans which attract and bind ε-amino groups of lysine in lipoproteins. This situation also decreases the ability of heart and arteries muscles to contract less and increases risk of heart failure.

Thus, all these mechanisms of CVD are directly linked with elevated level of Hcy. Along with the direct link, increased Hcy concentration also modulate other risk factors of CVD by the similar or different processes.

Hcy as dependent factor of CVD:

By aggravating hypertension and diabetes, Hcy act as a dependent factor in pathogenesis of CVD.

Hypertension: Hypertensive patients typically have higher Hcy than normotensive patients (Rodrigo *et al*, 2003). Each 5μmol/L increase in plasma Hcy may increase systolic and diastolic blood pressure as 0.7/0.5 and 1.2/0.7mmHg in men and women respectively, which is also independent from renal function and B-complex vitamins status (Lim & Cassano, 2002). The mechanisms which are induced by elevated levels of Hcy in hypertension are (Stehouwer *et al*, 2005):

- Hcy-induced arteriolar constriction,
- Renal dysfunction and
- Increased arterial stiffness leads to hypertension

Type-2 Diabetes: For each 5μmol/L increase in serum tHcy, mortality may raise by 17% in non-diabetics and 60% in diabetic subjects (Hoogeveen, 2000)**.**

Hyperhomocysteinemia may lead to diabetes by:

- Elicitation of oxidative stress, systemic inflammation, and/or endothelial dysfunction. These factors are known to promote insulin resistance and β-cells dysfunction, which are two important underlying causes for type 2 diabetes (Song *et al*, 2009).

- Methylenetetrahydrofolate reductase (*MTHFR*) is shown to act synergistically with angiotensin-I-converting enzyme (*ACE*) to modulate type 2 diabetes risks. The C677T polymorphism (rs1801133) of *MTHFR* is the most studied genetic variation and is associated with hyperhomocysteinemia. Many studies have also reported association of C677T with type 2 diabetes and related complications (Mehri *et al*, 2010).

Suggestion to decrease Hcy levels: The therapeutic options for lowering elevated Hcy are (Lonn *et al*, 2006; Steenge *et al*, 2003 and Schnyder *et al*, 2001):

Folic acid: 500-5000µg	Choline: 250-3000mg
Vitamin B6: 10-500 mg	Inositol: 250- 1000mg
Vitamin B12: 1000-3000µg	Zinc: 30-90mg
Trimethylglycine (TMG): 500-900mg	S-adenosyl-methionine: 200- 800mg

1. Folate supplementation (0.5 to 5mg/day) significantly reduces tHcy concentration by 25% in patients with mild to moderate hyperhomocysteinemia. Supplementation with vitamin B_{12} produces a small additional effect (7%), whereas vitamin B_6 treatment alone only reduces post-methionine load concentrations of tHcy (Xu *et al*, 2008).
2. Betaine (trimethyl glycine) reduces fasting Hcy by 12% to 20% without altering folate levels. Choline, a precursor to betaine, decreases fasting and post methionine load concentration of Hcy. Both betaine and choline also have an adverse impact on blood lipid profile (Joshi, 2006).
3. Daily supplementation of a novel drink powder (4gbetaine, 800µg folic acid, 5.2µg vitamin B_{12}, and 2.8mg vitamin B_2) or a multiple micronutrients tablet supplementation to pregnant women for 12 weeks may significantly reduce mean plasma Hcy level by 23.6% (James *et al*, 2019).

CONCLUSION

Hcy is a natural intermediate product of methionine synthesis. Production of Hcy by methionine is necessary for the metabolism of thiol compounds. Metabolism of Hcy is mainly dependent on B-complex vitamins status. The biological relevance of Hcy metabolism and its association with various dependent factors makes it an important etiological factor for cardio vascular diseases. So, it is better to control Hcy levels before it crosses the minimal therapeutic line (6.3µmol/litre). Inclusion of dietary sources of B-complex vitamins are the best option to use for management of elevated Hcy levels.

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