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Review

Homocysteine and cardiovascular disease

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Based on prospective and experimental data, mild to moderate elevation of homocysteine is a stabilized and independent risk factor for cardiovascular disease. The hyperhomocystenemia is a consequence of inhibition of transsulphuration pathway or inhibition of remethylation pathway of homocysteine metabolism, transsulphuration is mediated by CBS and remethylation is mediated directly by MS and indirectly by MTHFR. The SNPs in these genes alter the activity of corresponding proteins hence it may or may not be responsible for mild to moderate hyperhomocysteinemia. The consequences of hyperhomocysteinemia arise in the form of endothelial cell injury by increased oxidative stress and reduced bioavailability of nitric oxide, increased platelet adhesiveness, enhanced LDL deposition on arterial wall and activation of coagulation cascade. Some environmental factors are also known to contribute in progression toward disease.

Key words: Homocysteine, oxidative stress, inflammation.

INTRODUCTION

Cardiovascular disease is caused by disorders of the heart and blood vessels, and includes coronary heart disease (heart attacks), cerebrovascular disease (stroke), raised blood pressure (hypertension), peripheral artery disease, rheumatic heart disease, congenital heart disease and heart failure. An estimated 17 million people die from cardio vascular diseases (CVDs), particularly heart attacks and strokes every year, thus making stroke the second leading cause of death. Projections to the year 2020 indicated an increase in the number of CVD cases, majority of such cases will be from developing countries including India (WHO manual, 2002). Raised blood pressure is the most important cardiovascular risk factor, contributing to one half of the coronary heart diseases and approximately two third of the cerebro-vascular diseases because, it exerts excessive pressure on the interior wall of arteries, thereby damaging internal endothelial lining of the blood vessels and, at the damaged sites, homocysteine (Hcy) mediated enhanced lipid peroxidation and generation of free radicals result into inflammation (Libby et al., 2002). Due to developing inflammation, artery gets choked leading to partial to complete blockage of blood supply to the respective organ.

An increased homocysteine in the blood is thus related with acute endothelial dysfunction. Wilcken and Wilcken in 1976, Shown in the first clinical study supporting the theory that coronary artery disease (CAD) had a correlation with higher levels of homocystine. It has been demonstrated that in the presence of traditional risk factors, homocysteine may permissive role in endothelial damage.

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Abbreviations: Hyc, Homocysteine; **CAD,** cardio vascular disease; **MTHFR,** methylene tetrahydro folate reductase; **CBS,** cystathionine beta syntheses; **MS,** methinine synthase; **SAM,** S- adinosyl methonine; **SAH,** S- adinosyl homocysteine; **Tm,** transition metal; **SOD,** super oxide dismutase; **RS-H,** free thiol; **NO,** nitric oxide; **LDL,** low density lipid; **OxLDL,** oxidised low density lipid; **CP,** ceruloplasmin; **Cb,** cynocobalamine.

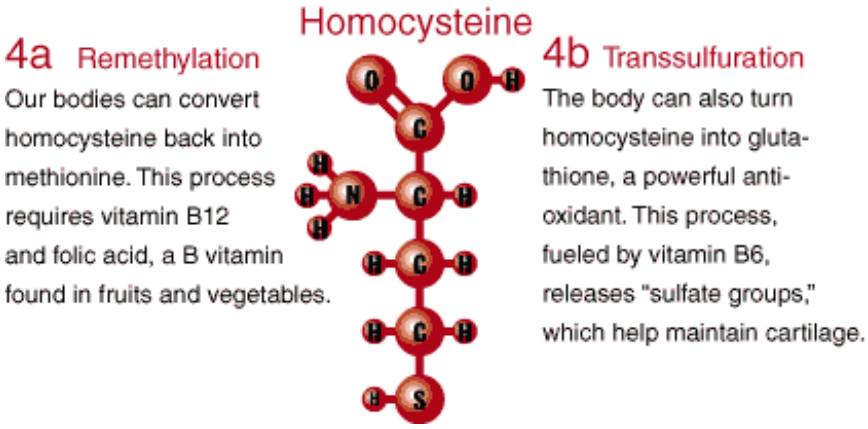

Figure 1. Homocysteine (Source: www.biopsychiatry.com/article/sameart.htm.).

THE HOMOCYSTEINE

The homocysteine is a sulphur containing amino acid (Figure 1) which is not a part of diet, it is synthesized from methionine, an essential amino acid.

THE METABOLIC PATHWAY OF HOMOCYSTEINE

Homocysteine is produced from methionine as a product of a large number of transmethylation reactions dependent on S-adenosylomethionine.

Homocysteine metabolism

Activation of methionine by ATP in the presence of methionine S-adenosyl-transferase, S-adenosylmethionine (SAM) demethylation into S-adenosylhomocysteine (SAH) coupled with methylation of an acceptor R into RCH3. Hydrolysis of SAH into homocysteine (HCYS) and adenosine catalyzed by S-adenosylhomocysteine hydrolase. Condensation of serine (Ser) with HCYS to form cystathionine (CTT) by cystathionine b-synthase (CBS). N5-methyltetrahydrofolate (5-methylTHF) demethylation into THF and HCYS remethylation into Methionine by methionine synthase (MS). Reduction of 5,10-methyleneTHF into 5-methylTHF catalyzed by N5,10-methylenetetrahydrofolate reductase (MTHFR). As shown in Figure 2 the three enzymes contributed to homocysteine metabolism, when there is an excess of methionine ingested, homocysteine follows the transulphuration pathway, through which homocysteine is converted automatically to cysteine. The first reaction in this pathway is catalyzed by vitamin B6 dependent enzyme cystathionine β synthase (CBS) (Finkelstein et al., 1990). Under conditions with a negative methionine balance homocysteine follows another pathway in which, the homocysteine is remethyleted into methionine and this step is catalyzed by methionine synthase (MS), which uses B_{12} as coenzyme and methylene-tetrahydrofolate (MTHF) as substrate.

The formation of MTHF from tetrahydrofolate is catalyzed by Methylene-tetrahydrofolate reductase (MTHFR) (Engbersen et al., 1995).

BLOOD HOMOCYSTEINE CONCENTRATION

Under normal metabolic circumstances, there is a strict balance between homocysteine formation and elimination. However, under the conditions of reduced or total loss of activity of homocystine metabolizing enzyme due to mutation in corresponding gene, this metabolic balance is disturbed and resulting into hyperhomocystenimia (Ueland et al., 1993). The increased level of homocysteine (Table 1) (30 to 100 µmol/L and >100 µmol/L) from normal (15 to 30 µmol/ L), is associated with CVDs (Kang et al., 1992). The moderate hyperhomocysteinemia has been identified as a new independent risk factor for cardiovascular disease. This fact has developed interest in the study of genetic variants involved in homocysteine metabolism and its relationship to pathogenesis.

THE HYPERHOMOCYSTEINE BASIS OF CARDIOVASCULAR DISEASE

Homocysteine is a sulfur-containing amino acid, $COOHCH(CH_2CH_2SH)NH_3$, that is formed during methionine metabolism. As a free amino acid, it exists in either the reduced (homocysteine, a thiol) or oxidized (homocystine, a disulfide RSSR) form. Its redox chemistry is dominated by its thiol group (SH), which in contrast to most nucleophiles is readily oxidized. Oxidation of two homocysteine molecules yields the disulfide, two protons (H+) and two electrons (e-).

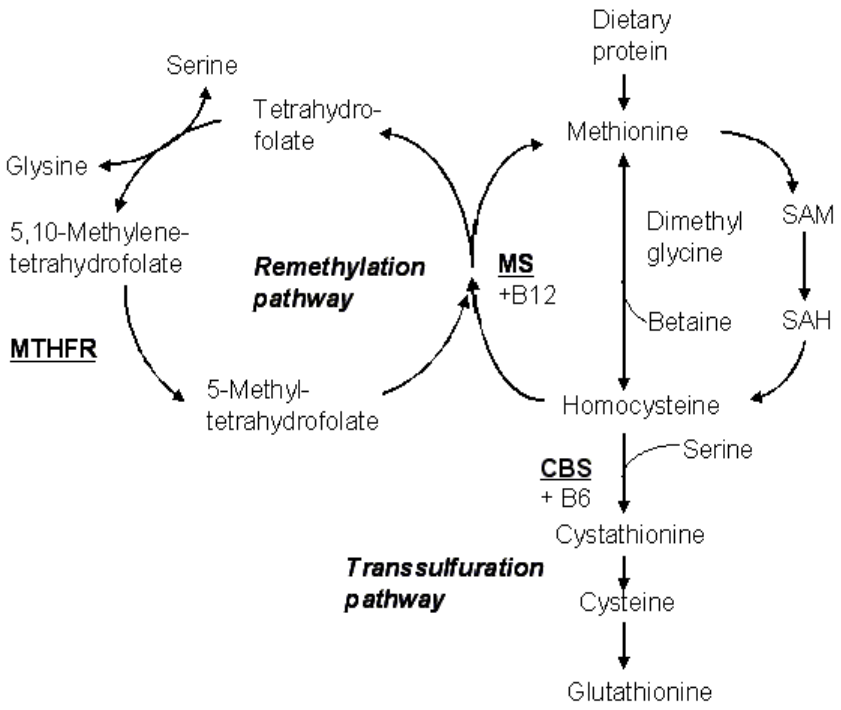

Figure 2. The metabolic pathway of homocysteine.

Table 1. Blood reference ranges for homocysteine.

Sex	Age (years)	Lower limit	Upper limit	Unit	Elevated	Therapeutic target (mg/L)
Female	12 to 19	3.3	7.2	µmol/L	> 10.4	< 6.3 (0.85)
	> 60	4.9	11.6	µmol/L		
Male	12 to 19	4.3	9.9	µmol/L		
	> 60	5.9	15.3	µmol/L	>11.4	< 6.3 (0.85)

Source: The Doctor's Doctor: Homocysteine.

2 RSH = RSSR + 2 H+ + 2 e-

In the presence of metal ions and oxygen, it can autooxidize, generating highly reactive partially reduced oxygen species such as superoxide and hydrogen peroxide (Ian F et al., 2000). Thiols also can initiate lipid peroxidation, produce hydroxyl radical, and oxidatively cleave proteins in a reaction that requires iron. Therefore, regulating the oxidation state of sulfur-containing amino acids is an important strategy for curbing cellular damage. The practical considerations suggest that reduced homocysteine may be capable of generating reactive oxygen species by autoxidation in the presence of transition metals. Autoxidation of homocysteine put active autoxidation of a free thiol (RS-H) such as homocysteine in the presence of transition metals, Tm, and superoxide dismutase (SOD).

The superoxide produced from the previous reaction can react readily with nitric oxide (NO) to form the oxidant peroxynitraite ($^{-}$OONO). This reaction is five-fold faster than the dismutation of superoxide by superoxide dismutase (SOD) makes reduced bioavailability of NO for endothelial cells. The endothelium-derived NO is able to mediate most of the anti-atherothrombotic functions of the endothelium. Therefore, a reduction in the bioavailability of NO constitutes an important step in the pathobiology of atherosclerotic vascular disease. Clinical studies have shown that patients with hypertension have a blunted arterial vasodilatory response to infusion of endothelium-dependent vasodilators and that inhibition of NO raises blood pressure (Hermann et al. 2006).

The increased concentration of homocysteine auto-oxidized

with trace metal ions, generating reactive oxygen species such as superoxide anion, hydrogen peroxide, hydroxyl, and thiol free radicals (Munday, 1989; Schöneich et al., 1989), which is able to reduce Cu^{2+} into Cu+ of ceruloplasmin. Cu^{2+}-Ceruloplasmin has a protective effect on LDL oxidase. Thus one may assume that HCY may activate the LDL oxidase property of CP by reducing the redox-active Cu^{2+} of the protein. HCY up to 100 µmol/l stimulated LDL oxidation in presence of CP inside the arteries (Heinecke et al., 1987; Hirano et al., 1994; Wood and Graham, 1995). Oxidized LDL has many characteristics that potentially promote atherogenesis, in addition to the ability to be taken up rapidly by macrophages to form foam (cellsFoam cells are not dangerous as such, but can become a problem when they accumulate at particular foci thus creating a necrotic centre of the atherosclerosis). LDL is a chemo-attractant for circulating monocytes (Quinn et al., 1987).

The chemo-attractant activity of LDL resides in its lipid moiety, and is attributable to lysophosphatidylcholine generation during the conversion of LDL into its oxidized form. OxLDL promotes the differentiation of monocytes into tissue macrophages by enhancing the release of macrophage colony stimulating factor from endothelial cells (Rajawashishath et al., 1990), and inhibits the motility of resident macrophages (Quinn et al., 1987). Colony stimulating factor is a chemo-attractant for Tcells (Mc Murray et al., 1993), and consequently the atherosclerotic plaque contains primarily monocytes and T cells. Unlike native LDL, oxLDL is immunogenic (Polinski et al., 1989), and it is also cytotoxic to various cell types, including endothelial cells (Hessler et al., 1983), resulting in loss of endothelial integrity. OxLDL also activates matrixdigesting enzymes, which may play a role in plaque instability (Xu et al., 1999).

THE CASE – CONTROL STUDY – EVIDENCE

The case control studies are those which consider a factor being studied, for which the cases (patients) and the controls (healthy) are differ but other factors are strictly similar, to find out the contribution of the factor being study in disease susceptibility. I have taken some random selected studies from developed and developing countries which are shown in Table 2. In approximately all ethnic groups are showing a significant positive correlation between homocysteine concentration and cardiovascular diseases.

Causes of hyperhomocystenimia

One or a combination of genetic, and life style factors causes modest elevations in homocysteine levels without associated homocystinuria.

Genetic factors

Enzyme deficiencies and mutations

i) Cystathionine b-synthase
ii) Methionine synthase
iii) Methylenetetrahydrofolate reductase

Recently, more than 15 different genes are under investigation for their relationship to plasma homocysteine levels. These three genes have been identified for hyperhomocystenimic condition in humans.

Methylenetetrahydrofolate reductase (MTHFR): Nine thermo stable mutations of the MTHFR gene, located on chromosome 1, inherited as an autosomal recessive trait, could be responsible for its reduced activity. Hyperhomocysteinemia is only observed in the homozygous state (Table 3). A common polymorphism (677C→T) in this gene has been shown to be associated with a decreased enzyme activity and consequent higher circulating levels of homocysteine. The C677T mutation of the MTHFR gene, which leads to the synthesis of a thermo labile form of MTHFR that is responsible for 50% of the MTHFR activity, has a homozygosity prevalence(Table 3), which may vary between 1.4 and 15% in the population, according to geographic area (D'Angelo and Selhub, 1997; Frosst et al., 1995; Kang et al., 1993; Kluijtmans et al., 1996).

In homozygous individuals, this autosomal recessive mutation provokes a moderate hyperhomocysteinemia because mutated enzyme has reduced binding with their substrate folete, this effect of mutation can be decreased by increased concentration of folic acid (Vitamin B9) in blood and no elevation of blood homocysteine has been observed when plasma folate concentrations are greater than 15.4 nmol/L (Jacques et al., 1996). Folate deficiency could be partially responsible for the expression of the MTHFR thermo labile genotype. It is hypothesized that the serum levels of homocysteine and/or the MTHFR polymorphism could influence the risk for coronary artery disease (CAD) (Frosst et al., 1995).

Thirty-three point mutations of the CBS (cystathionine β synthase) gene, which is located on chromosome 21, have been identified. Among these mutations, the G919A transition, leading to substitution of guanine by adenine in position 919, frequently encountered in the Irish population, is observed in subjects who are refractory to vitamin B6 treatment. The T833C mutation, frequent in the German population, is observed in vitamin B6 responders. People who are heterozygous for CBS deficiency (between 1 in 70 to 1 in 200 in the general population) have normal homocysteine levels in 30 to 50% of cases. Substitution of thymine in the place of cytosine at position 833 (833T→C) is the most frequent polymorphism of this gene (Table 3). Due to this substitution, the enzyme show

Table 2. Showing significantly incensement in homocysteine concentration between patient (case) and healthy (control).

	Case			Control			
Source	**Total No.**	**Mean age (Years)**	**Mean homocysteine SD (µmol/L)**	**Total No.**	**Mean age (Years)**	**Mean homocysteine SD (µmol/L)**	**Result (difference in plasma homocysteine level between case-patients and controls)**
De Kadziela et al. (2003); UK	120	59±9.6	12.8 ± 5.1	106	47.4 ± 6.0	10.0 ± 5.0	Significant
Pezzini et al. (2002); Italy	56	45	13.2 (7 - 32.8)	36	_	8.9 (5 - 17.3)	Significant
Gallai (2001); Italy	25	51.6	17.88	30	50.6	6.0	Significant
MacIlroy (2002); Ireland	63	73.8	15.2	71	74.3	10.7	Significant
Almawi et al. (2004).	96	55.3 ± 11.3	18.47 ± 3.73	404	50.7 ± 8.9	16.28 ± 4.16	Significant
Kerkeni et al. (2006).	100	NA	15.86 ± 8.63	120	NA	11.90 ± 3.25	Significant
Yingdong (2002); China	43	62	19.3	42	59	13.7	Significant
Yoo (2000); South Korea, Korea	122	74.2	12.3	217	72.2	10.2	Significant

Table 3. Genetic cause of hyperhomocystenemia.

The homocysteine metabolizing gene	**Effect on homocysteine metabolizing concentration**
MTHFR defect (Activity< 20%)	Homozygous mutation, rare in population Severe homocystenemia in fasting condition
MTHFR deficiency (Activity < 50%)	Homozygous C677T mutation, 10% in population Moderate basal hyperhomocystenemia
CBS defect (Activity < 2%)	Homozygous, rare in population Severe basal hyperhomocystenemia
CBS deficiency	Heterozygous Homocysteine concentration increase in postmethionine load
Functional methionine synthase deficiency.	Altered cobalamin metabolism (rare mutations), intermediate to severe basal hyperhomocysteinemia

Table 4. Non-genetic cause of homocystenemia.

Study	Factor	Effect	Result
Nygyard et al. (1995) Involved 7,591 Men and 8,585 women	**Gender** Male/female	Males showed a 1.82 uM higher homocysteine level than females.	The combined effect of age, sex, and smoking reached 4.8uM homocysteine difference.
	Age Older (65 - 67) Younger (40 - 42)	Older persons showed a 2.2 uM higher homocysteine level than younger persons.	Smoking is one of the major risk factors for cardiovascular disease.
	Smoking Smokers Non-smokers	Heavy smokers (>20 cigarettes per day) showed a 1.91 uM higher homocysteine level than non-smokers.	
Wouters et al.(1995)	**Menopause** Premenopausal Post-menopausal	Homocysteine levels were higher in postmenopausal females than premenopausal females.	–
Cravo et al. (1996)	**Alcohol** consumers/non-consumers	Homocysteine levels were also elevated two-fold in high alcohol consumers (> 1.5g/kg/day) compared to non-consumers.	–

irresponsiveness for their coenzyme PLP (pyridoxal phosphate) and reduce their activity to catalyze homocysteine, hence, pyridoxine treatment can decrease the hyperhomocysteinemia (McCully, 1983).

Five known mutations affect methylcobalamin synthesis, an essential cofactor of methionine synthase (Cbl E and G; Cbl C, D, and F) (Allen et al., 1993; Banerjee and Matthews, 1990; Fenton and Rosenberg, 1995; Rosenblatt, 1995). These mutations induce a functional deficiency in methionine synthase that leads to intermediate hyperhomocysteinemia and hypomethioninemia.

The functional deficiency depends on mutations in the methionine synthase gene or in the gene encoding methionine synthase reductase, an enzyme involved in the reductive activation of methionine synthase (Leclerc et al., 1998). This gene shows a common polymorphic form (2756A→G), base transition results into conversion of aspartic acid to glycine, changing the crucial binding site of coenzyme (Vitamin B12) and therefore might influence in the secondary structure with possible reduced functional activity (Table 3), and the function can restore with vitamin B12 treatment (Zhang and Dai, 2001).

Non-genetic factors that affect homocysteine level

Several non genetic (environmental and physiological factors) factors have been found to increase the severity of disease and/or risk factor for cardiovascular disease. I have selected one large cross-sectional study and two small studies which are based on case control method in following Table 4, that indicated the life style of individual increases risk of cardiovascular diseases as well as play additive role on risk level due to hyperhomocystenimia .

CONCLUSION

The impaired metabolism of homocysteine produces the condition of hyperhomocysteinemia which is an independent risk factor for cardiovascular disease because it exert negative role on endothelial membrane. The polymorphic variant of homocysteine metabolizing genes may contribute in hyper condition of homocysteine, but environmental factors may fluctuate this from positive site to negative site and vice versa.

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