PLASMA HOMOCYSTEINE: A DIAGNOSTIC MARKER IN VARIOUS DISORDERS

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ABSTRACT

Hyperhomocysteinemia is highly prevalent in world population both in developing and developed countries. Elevated plasma homocysteine level has been associated with an increased risk for numerous pathological conditions that is not related to conventional risk factors. Various nutritional, hormonal, and genetic factors that are associated with elevations in circulating homocysteine levels are also linked with particular disease conditions including cardiovascular disorders, cancer development, endocrine disorders, renal disorders, autoimmune diseases, bone metabolism and neurodegenerative diseases. Recently numerous other pathological conditions are reported to be linked with elevated serum homocysteine indicating further findings remains to be explored. The knowledge of the connection between disease, methyl imbalance and epigenetic control of gene expression has steadily progressed. However, homocysteine imbalance and its role in healthiness and diseased conditions are not clearly understood. Therefore, the current review highlights the understanding of elevated serum homocysteine level and its impact on particular disease manifestations.

KEYWORDS: homocysteine, hyperhomocysteinemia, diagnostic marker, cardiac marker, homocysteine metabolism.
INTRODUCTION
Homocysteine is sulfur containing amino acid produced from the catabolism of essential amino acid, methionine and remethylated to methionine in the presence of methyl synthase using vitamin-B12 or cyanocobalamin as cofactor and methylene tetrahydrofolate reductase as a cosubstrate. It can be irreversibly degraded by cysthionine beta-synthase requiring vitamin-B-6 as a cofactor. Homocysteine is composed of reduced, oxidized and oxidative mixed disulphide form, both free and bound proteins.[1] In other word we can say that homocysteine is an intermediate product of methionine metabolism and is itself metabolized by 2 pathways (Figure 1).

• The remethylation pathway which regenerates methionine and trimethylglycine (TMG). These methyl groups can be transferred to homocysteine to remethylate it back to methionine and S-adenosylmethionine (SAM). The detoxification or remethylation requires following minimum factors - 1) Folic acid 2) Vitamin B12 3) Zinc and 4) TMG.
• The transsulphuration pathway which degrades homocysteine into cysteine and then taurine (this pathway is dependent on vitamin B6). This fact is evident from the higher amount of vitamin B6 needed by red meat and chicken eaters to diminish the homocysteine levels in a safe range.

Since homocysteine exists as an intermediate metabolite and located at a critical metabolic crossroad, therefore it has a direct and indirect impact on both i.e. on methyl and sulphur groups’ metabolism occurring in the body. Experiments have demonstrated that all methylation reactions are completely inhibited if high enough levels of homocysteine and adenosine accumulate in the cell.[2] The accumulation of homocysteine in the cell has its implications as altered metabolism and thus has pathological and clinical significances. Further, nutritional deficiencies of vitamin B6, vitamin B12 and folic acid can also produce mild to moderate elevations of plasma homocysteine level. In this view this, higher homocysteine levels is a good candidate for the term “toxic endometabolite” clinically described as hyperhomocystinemia.

Hyperhomocystinemia has received increasing attention during past few decades and has been associated with smoking, dislipidemia, hypertension and obesity as independent risk factors for cardiovascular diseases. In addition to this, increased homocysteine levels have also been implicated in neural tube defects, spontaneous abortions, placental abruption, low
birth weight, renal failure, insulin dependent diabetes mellitus and complications of diabetes, neuropsychiatric disorders, cancer biology and cardiovascular disorders.[3]

Ample of evidences in healthy men and women suggests that certain acquired and genetic determinants enhance the total plasma homocysteine level. Women tend to have lower basal levels than men.[4] Westernized adult black South Africans have shown 45% lower homocysteine levels than in aged matched white adult indicated the role of genetic differences in homocysteine metabolism.[5] The individuals in the lowest quartiles for serum folate and vitamin B12 have significantly higher concentrations of homocysteine. Increased homocysteine concentration has been reported in men with lowest quartile of serum pyridoxal 5'-phosphate.[6] Additionally hyperhomocysteinemia has been reported to be associated with chronic alcohol consumption, cigarette smoking and high coffee intake.[7,8]

Furthermore, homocysteine plays a central role in sulphur and methyl group metabolism and elevated levels would be expected to negatively effect the biosynthesis of SAM, carnitine, chondroitine sulfates, coenzyme A, coenzyme10, creatine, dimethylglycine, epinephrine, glucosamine sulfate, glutathione, glycine, methionine, pantethine, phosphatidylserine, serine and taurine.[3] Therefore, it could be suggested that high homocysteine level directly or indirectly known to be involved in deoxyribose nucleic acid (DNA) methylation, growth processes, signal transduction and detoxification reactions.

Figure: 1 Key component of the homocysteine, remethylation and trans sulphuration pathway and homocysteine metabolism. THF, tetrahydrofolate; MTHFR, methylene tetrahydrofolate reductase; and CBS, cystathionine beta synthase.
HYPERHOMOCYSTEINEMIA AND ASSOCIATED DISEASES

Cancer: Inspite of being a risk factor for atherosclerotic cardiovascular diseases, recent research suggest homocysteine acts as a marker for cancer. High growth rate, a characteristic of malignant cells, requires higher methionine due to increased protein synthesis and transmethylation reactions. Normal cells meet their methionine requirement by synthesizing it from homocysteine. Contrary to this, methionine-dependent malignant cells in organs such as the lung, kidney, breast, colon and bladder cannot convert homocysteine to methionine, which results in hyperhomocysteinemia. Hyperhomocysteinemia is also associated with folate deficiency because folate cofactors act as essential intermediates in homocysteine remethylation, S-adenosylmethionine (SAM) synthesis (which is the most important methyl donor) and in the production of purine and thymidine for DNA synthesis. If these alterations are not corrected by DNA repair enzymes, normal control of gene expression (turning genes off and on) can be lost resulting in cancer. Normally these changes are then heritable in the cells or tissues, where the mutation takes place. Many studies have shown that epigenetic events, which don’t change the sequence of nucleotides but modify the DNA by altering its methylation pattern, can also be deleterious and result in cancer.

DNA methylation involves transfer of a single carbon from SAM specify short DNA nucleotide sequences and is catalyzed by one of the DNA methyltransferase enzyme. Research has shown that improper DNA methylation (not enough or too much) can either activate or inactivate a gene. Simply, if certain genes are activated when they are supposed to be inactive or, vice versa, inactivated when they are supposed to be active cancer can result.

It has been reported that rapid proliferation of tumor cell may results in elevated total homocysteine concentration that reflects the number of live cells. An elevated homocysteine level is thought to contribute to the pathogenesis of a number of chronic disorders including atherosclerosis, thromboembolism, stroke, osteoporosis, recurrent miscarriages and other complications of pregnancy as well as cognitive impairment including dementia and Alzheimer’s disease. Thus serum homocysteine may be a potentially useful tumor marker to monitor tumor activity. Wu et al. suggested that plasma homocysteine levels serves as a marker for tumor.

Researchers from the University of Utah measured total homocysteine level in the blood of cancer patients undergoing treatment. Increased homocysteine coincided with the elevations
in the certain tumor markers. It was believed that the rapid proliferation of tumor cells was parallel to the higher concentrations of circulating homocysteine. Tumor markers also increase in the event. However, homocysteine would decrease in response to the tumor cell death. The researchers speculated that the elevations in the homocysteine level could be secondary to several biochemical factors including folate deficiency, oxidative stress or aberrations in DNA metabolism. These factors supposed to be elevating latter could also be the reason behind elevated carcinogenesis. These lines confer that homocysteine can be used as a better tumor marker for monitoring during treatment. Serum homocysteine was strongly and significantly predictive of invasive cervical cancer risk. This association could reflect folate, vitamin B₁₂ and /or vitamin B₆ inadequacy or genetic polymorphisms affecting one carbon metabolism.[15]

Several biochemical changes have been identified in association with hyperhomocysteinemia indicating that elevated total homocysteine in blood circulation created a risk for cancer and it is likely that hyperhomocysteinemia is a risk factor for carcinogenesis. The effect of folate on DNA methylation has been demonstrated by Piyathilake et al, in squamous cell carcinoma (SSC) as well as in tissues at risk of developing a SSC through methylation of DNA, another consequence of hyperhomocysteinemia.[16]

In addition to gene alterations, perturbations in DNA methylation patterns (epigenetic changes), including both local hypermethylation and genome wide hypomethylation are frequently observed early in tumorigenesis. Therefore, genomic instability, including genetic and/or epigenetic alterations may be the first step in carcinogenesis. Knowledge of these biochemical mechanisms is likely to lead to more effective cancer diagnosis and therapy.[17] The homocysteine level was inversely correlated with folate status in cancer cases and tended to be higher in patients with methylenetetrahydrofolate reductase 677TT (MTHFR 677TT) genotype. These results suggest an important role of homocysteine in breast tumorigenesis. The folate level was an independent predictor of hyperhomocysteinemia in patients with breast cancer.[18]

**Cardiovascular diseases**

With respect to the normal plasma homocysteine levels (5-15 µm/L), increased level correlates with significant increase in risk of coronary artery diseases (CAD),[19,20], myocardial infarction,[21] , stroke,[22,23] , peripheral occlusive diseases,[24] cerebral occlusive diseases.[25] and retinal vascular occlusion.[26] Another study reported that elevated levels of
homocysteine above 12.1 μmol/L may double the risk of pathophysiological conditions such as atherosclerosis, myocardial infarction, and peripheral vascular diseases.\[27\] In a study of 304 patients with CAD versus controls, Robinson et al, found the odds ratio for CAD increased as plasma homocysteine increased, even within the normal range. A 5 micromolar/litre increase in plasma homocysteine was correlated with a specific increase in the odds ratio of 2.4 (P<0.001), with no “threshold effect”.\[28\] Homocysteine facilitates the generation of hydrogen peroxide by creating oxidative damage of low density lipoprotein (LDL) cholesterol and endothelial cell membranes.\[29\] Hydrogen peroxide can then catalyze injury to vascular endothelium.\[29\] Elevated homocysteine levels have been established as an independent risk factor for intermittent claudication (IC) and deep vein thrombosis. Elevated homocysteine levels corresponded with increased incidence of IC and decreased serum folate levels in a study of 78 patients with IC.\[30\] Group of researchers in Netherland reported high homocysteine levels as a significant risk factor for deep vein thrombosis, with a stronger relationship among women than men.\[31\] Epidemiological data suggest that homocysteine levels above 6.3 cause a steep progressive risk of heart attack.\[28\] Other study found that each 3-units increase in homocysteine equals a 33% increase in myocardial infarction risk.\[32\] In one prospective study, the risk of vascular disease was raised 1% for every 1 μmol/L increase in total plasma homocysteine concentration.\[33\] The role of modest elevation in homocysteine without associated homocystinuria in the causation of atherosclerotic and thromboembolic vascular diseases has been investigated. Such modest elevations in plasma homocysteine can be related to genetic, physiologic, pathologic, and nutritional factors. Thus, heterozygous methylene tetrahydrofolate reductase (MTHFR) mutations (e.g. thermolabile MTHFR), age, gender, post menopausal status in women, smoking, sedentary lifestyle, dietary factors including increased intake of animal protein, which has a higher methionine content and low intake of folate, vitamin B6 and vitamin B12, renal failure, transplantation and medications such as corticosteroids and cyclosporine have been associated with hyperhomocysteinemia. Recently a meta-analysis reported that the elevated homocysteine levels are an independent predictor for subsequent cardiovascular mortality or all cause mortality, and the risks were more pronounced among elderly people.\[34\]

The potential mechanism of athero and thrombogenicity associated with elevated level of homocysteine include.\[35\] i). Endothelial dysfunction related to direct endothelial cell damage and impaired production of nitric oxide. In addition to this hyperhomocysteinemia may cause diastolic dysfunction of vessels and reduction of flexibility due to its influence on vascular
wall remodelling. These mechanisms may lead to an increase in blood pressure and strengthen the development of hypertension and damage body organs in patients with this disease.\[36\] ii). Stimulation of smooth muscles cell proliferation. iii). Lipid abnormalities, including increased plasma triglycerides and susceptibility to oxidation of LDL. iv). Increased thrombogenicity mediated by promoting the adhesion of platelets and release of platelet derived growth factors due to homocysteine induced endothelial damage, activation of factor V and factor Xa. v). Inhibition of protein C activation, inhibition of cell surface expression of thrombomodulin and decreased tissue plasminogen activator (TPA) activity.

Table 1: Serum homocysteine and risk of coronary artery disease.\[28\]

<table>
<thead>
<tr>
<th>RISK OF CORONARY ARTERY DISEASE</th>
<th>Serum homocysteine (µmoles/liter of the blood)</th>
<th>Percentage of individuals in this range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower risk</td>
<td>0-6.3</td>
<td>38%</td>
</tr>
<tr>
<td>Moderate risk</td>
<td>6.3-10</td>
<td>52%</td>
</tr>
<tr>
<td>Highest risk</td>
<td>Over 10</td>
<td>10%</td>
</tr>
</tbody>
</table>

In a very recent study homocysteine in older people who are at risk of coronary heart disease (CHD), as well as those with high homocysteine are at highest risk for fatal and nonfatal CHD.\[37\]

Diabetes

Okada et al, reported strong association between atherosclerosis, hyperhomocysteinemia and type 2 diabetes in the Japanese population.\[38\] Both insulin dependent diabetes mellitus and non insulin dependent diabetes mellitus progress to hyperhomocysteinemia in conjunction with compromised renal function.\[39\] Insulin resistance is the fundamental abnormality in the pathogenesis of type 2 diabetes, may lead to arterial damage through toxic effects of hyperinsulinemia. However, hyperhomocysteinemia is a characteristic finding in diabetic subjects with microalbuminuria and renal failure. Hyperhomocysteinemia is associated with insulin resistance, obesity and hypertension, as shown by the Framingham Offspring Study.\[40\] Drzewoski et al, reported that diabetic patients with bad metabolic control (HbA1c 9.8%) had significantly higher homocysteine levels in comparison to diabetics with normal HbA1c levels (6.6%).\[41\] Buysschaert et al, reported a higher incidence of complications that includes macroangiopathy and nephropathy in diabetic population in the group with hyperhomocysteinemia in comparison to the patients with normal homocysteine level.\[42\] Studies have reported elevated homocysteine levels in patients with diabetes mellitus and the presence of microvascular complications, nephropathy and retinopathy.\[43,44\] Both acute and
prolonged exposure to homocysteine had been resulted in detrimental effects on beta cell glucose metabolism, insulin secretory responsiveness and cell viability. Insulin has been shown to inhibit the irreversible metabolism of homocysteine into cysteine through the trans-sulfuration pathway when methionine is in demand. Homocysteine generates ROS in a redox-cycling reaction that explains the decline in viability of insulin-secreting cells, leading to reduced glucokinase phosphorylating ability, diminished insulin secretory responsiveness and cell death. Genetically inherited defects are the most important determinants of elevated levels of homocysteine. Recently elevated HbA1c was reported in 65.5% hyperhomocysteinemic diabetic patients. Recently, Huang et al, in a meta analysis study reported strong evidence on the causal association of raised homocysteine level with the development of diabetes mellitus type2. Strong evidence suggested that excess of plasma homocysteine disturb lipid metabolism via the oxidation of LDL particle and its aggregation, and enhancing atherosclerosis progression. Another line of evidence suggested that thiolactonase activity was affected in diabetics partly by a glycation process that accentuates the endothelial damages of homocysteine. Apart from the known and modifiable traditional risk factors in relation with diabetic retinopathy some newly emerging modifiable risk factors has been addressed in the field of biomedical research and hyperhomocysteaemia is one of them.

Renal diseases
It has been known for many years that the metabolism of homocysteine and other sulfur-containing amino acids are deranged in chronic renal insufficiency and end stage renal disease. Evidence has now accumulated showing that an elevated plasma homocysteine level is a risk factor for vascular diseases in both normal patients and those with renal disease. Hyperhomocysteinemia, occurs already at a glomerular filtration rate (GFR) of about 60 ml/min and when end stage renal disease has been reached. The prevalence of hyperhomocysteinemia is 85-100% in patients with renal disease. Homocysteine level increases as the renal function declines and progresses to end stage renal disease (ESRD), with the vast majority (85%) of dialysis patients ultimately experiencing mild-to-moderate hyperhomocysteinemia. The association between plasma homocysteine and GFR seems to be in linear and is present even in the range of hyperfiltration.

Hyperhomocysteinemia, the state of elevated plasma homocysteine levels, is very common among patients with chronic renal insufficiency and occurs almost uniformly in the ESRD
patients. Evaluation of plasma concentrations of the various cofactors and homocysteine metabolic substrates, and the effects of different therapies suggest that an abnormal folate metabolism may be the cause of hyperhomocysteinemia in uremic patients. In line with this, other findings supported that homocysteine remethylation, as assessed by stable isotope techniques, is impaired in dialysis patients. Increase in homocysteine level might lower renal function reported in the NAME study. Interestingly studies have reported that homocysteine is only a marker that is affected by renal dysfunction. Patients with kidney disease, for who exceptionally high rates of cardiovascular morbidity and death are observed; exhibit disproportionately elevated plasma homocysteine levels. Homocysteine levels increase as renal function declines and patients become increasingly refractory to the usual homocysteine lowering therapies. A recent study conducted in a Taiwan Chinese population reported that subjects with elevated homocysteine levels were older and had higher body mass index, blood pressures, fasting plasma glucose, total cholesterol, triglycerides, and estimated glomerular filtration rate (eGFR) than those with normal serum homocysteine level. The study findings concluded that elevated serum homocysteine levels appear to be closely associated with chronic kidney disease (CKD), while serum homocysteine levels are negatively associated with eGFR.

**Neurodegenerative diseases**

Elevated plasma homocysteine concentrations have been associated with dementia, depression and dyskinesia in Parkinson’s patients; however, determination of homocysteine concentrations alone was not sufficient for prognosis. Data from follow-up studies confirmed an independent role for homocysteine in predicting dementia. Further evidence suggests that depression is often related to vitamin B12 deficiency. Homocysteine is a surrogate marker for vitamin B deficiency, and in addition it also appears to predict the occurrence and progression of dementia. The Hordaland study of older men and women in Norway reported that the elevated homocysteine coupled with the T/T allele of the MTHFR gene was associated with depression. Recently, Parkinson patients who exercised regularly were shown to completely avoid an increase in plasma homocysteine concentrations after L-DOPA treatment, in contrast to sedentary Parkinson patients. Reduction of plasma homocysteine concentration remains a primary goal in controlling the symptoms of Parkinson’s disease.
Homocysteine can also complicate the progression of Alzheimer’s disease (AD). Increased plasma homocysteine levels have been described as a strong and an independent risk factor for Alzheimer’s disease. In newly diagnosed AD patients the rate of cognitive decline positively correlated with the concentration of plasma homocysteine. Patients with moderate AD and elevated homocysteine concentrations experienced greater behavioral disturbances associated with major depressive disorder. In a population study of more than 1200 Swedish women, a high plasma homocysteine concentration in group of middle aged patients was found as an independent risk factor for later dementia and Alzheimer’s disease. Recent study found hyperhomocysteinemia is a risk factor for AD in an Algerian population which is also associated with vitamin B12 deficiency. Available evidences suggest that plasma homocysteine concentrations may be used as a valuable prognostic and predictive biomarker for neurodegenerative diseases.

**Bone disorder**

The collective processes such as remethylation, transmethylation and trans-sulphuration; involving folic acid (co-factor in one carbon transfer reaction), vitamin B12 (coenzyme in the methyl transfer from 5-methyltetrahydrofolate to homocysteine) and vitamin B6 (cofactor in the trans-sulphonation pathway) are involved in the maintenance of homocysteine homeostasis. Several studies have reported that significant reduction in bone mineral density (BMD) and increased fracture risk in elderly people with elevated homocysteine concentrations and low vitamin B12 and folate status. In addition to this, clinical studies have reported the raised plasma homocysteine level (>15 μmol/L) in 30–50% of people older than 60 years. Multifactorial causes such as combination of environmental and genetic factors, nutrition, lifestyle, and hormonal factors have been suggested for hyperhomocysteinemia. Further, hyperhomocysteinemia may contribute to the development of osteoporosis. Vitamin B12 is closely correlated to homocysteine. The major determinants of homocysteine metabolism are vitamin B12 and folate. It has been reported that increased homocysteine level appears to be a strong and independent risk factor for osteoporotic fractures in older men and women. Supplementation with vitamin B12 and folic acid has been shown to be effective in normalizing homocysteine levels. Interestingly, the reversal of elevated homocysteine levels through folic acid and vitamin B12 supplementation prevent the problem of impaired bone health and osteoporosis. Thus, estimation of blood homocysteine level itself serves as a marker for bone turnover.
CRITICAL NEED FOR TESTING OF BLOOD HOMOCYSTEINE LEVEL
Measurement of blood levels of homocysteine provides a way to therapeutic approach. Those with a familial history of heart disease, stroke or AD are at high risk of elevated homocysteine level. Individuals that are at risk for developing cardiovascular disease are certainly candidates for homocysteine testing. Hence, if there is a strong family history of cardiovascular disease, then those individuals should be looked very carefully. The surveillance of toxic homocysteine and the proportional vitamins needed to detoxify it can be elucidated. Although detoxification via vitamins and TMG or choline can bring homocysteine levels in safe range but it is not always true. Homocysteine evaluation may serve to identify diabetic patients predisposed to sight threatening complication who may benefit from intensified screening and treatment strategy, including vitamin B6, vitamin B12 and folic acid supplementation at diagnosis, from where we can try to revert, limit or prevent the incidence and progression of diabetic retinopathy. Homocysteine is actually a risk factor for cognitive dysfunctions, dementia and AD. Therefore, testing homocysteine level in the elderly population should be done as well, keep in mind that homocysteine is a biomarker of folate and vitamin B12 deficiency. The individuals suspected of having folate and vitamin B12 deficiency should probably be tested, and actually should be tested for elevated total plasma homocysteine concentration. Special consideration should be taken while testing for plasma homocysteine level as various factors such as end-stage renal disease, hypothyroidism, psoriasis and estrogen deficiency can elevate homocysteine levels. Use of tobacco and medications such as phenytoin, sulfasalazine and methotrexate also can raise homocysteine levels. Other causes of hyperhomocysteinemia include gastric atrophy, inflammatory bowel disease and laxative use, all of which interfere with absorption of nutrients.

CONCLUSIONS
Newer scientific evidence indicates that there is no safe range for homocysteine. While some epidemiological data revealed that levels above 6.3 are hazardous. Elevated concentrations of homocysteine in blood are characteristic of a number of pathological conditions; pointing to the thought that homocysteine could be one of the diagnostic markers for those conditions, thus, homocysteine management represents an important goal for optimizing health. It remains unclear whether elevated homocysteine concentration directly contributes to the pathogenesis of disease or represents a biomarker of metabolic aberrations. However, it is clear from dietary and genetic animal models of hyperhomocysteinemia that well defined
adverse outcomes, such as vascular dysfunction, can be observed. Hyperhomocysteinemia is detected in patients with diabetes mellitus which may contribute to the development of diabetic complications. This review warrants the investigation of influence of diabetic treatment on homocysteine levels. Intervention strategies to reduce plasma homocysteine concentrations have met with mixed results, not just in the case of vascular disease, but also with respect to neurodegenerative disorders, diabetes and renal health. Therefore, a more precise understanding of the relationship between homocysteine balance and disease remains an important area of investigation, particularly for old age and for those populations that may be at the greatest risk of hyperhomocysteinemia.

REFERENCES


