Hyperhomocysteinemia: A Risk of CVD

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Abstract
Homocysteine, a sulfur containing amino acid formed from methionine, an essential amino acid especially abundant in animal proteins. High blood level of homocysteine is called hyperhomocysteinemia indicates altered normal metabolism of homocysteine. The amino acids homocysteine has been identified as a new independent risk factor for cardiovascular diseases in many studies Hyperhomocysteinemia in blood can be cause by many factors; most common cause has been either due to the deficiency of nutritional cofactors or genetic defects in production of enzyme required for metabolism of homocysteine. Vitamin B6, B12 folic acid are nutritional cofactors required for proper functioning of the methionine cycle improve methylation and may protect the atherosclerosis.

In this review, metabolism of homocysteine, role of homocysteinemia on CVD and possible nutritional intervention for lowering homocysteine has been discussed. This paper provides an updated summary of the published studies on relation of plasma total homocysteine with risk of cardiovascular disease. It was well proven from many epidemiological studies that elevated homocysteine is an independent risk for atherosclerosis and CVD. Although the major studies have reported vitamin cofactors folate, vitamin B12, B6 supplementation significantly decreases homocysteine levels, but its effect on lowering cardiovascular risk is controversial. Further research need to explore the role of nutritional cofactors vitamin folate, B12, B6 in lowering the risk of CVD.

Keywords:- Homocysteinemias, CVD, methionine, atherosclerosis..

1. Introduction
Cardiovascular diseases (CVD) are one of the leading causes of death worldwide. The Indian subcontinent (including India, Pakistan, Bangladesh, Sri Lanka, and Nepal) are among the highest rates of cardiovascular disease (CVD) globally [1]. Hyperhomocysteinemia (HHCy) has been emerged as a new independent risk factor for CVD. Hyperhomocysteinemia is a medical condition of abnormal high level of homocysteine (above 15µmol/L) in serum [2]. Homocysteine (Hcy) is a non-protein α amino acid, produced in the normal metabolism of methionine. Methionine is an essential amino acid, abundant in animal protein. Homocysteine is biosynthesized from methionine through S-adenosyl methionine [3].

Methionine →S-adenosyl methionine →homocysteine
About 50% of homocysteine is remethylated to methionine via methione synthase enzymatic pathway, require vitamin B12 and folate as methyl donor or other methylaton pathway require betaine as methyl donor. In presence of sufficient methionine, homocysteine converted into cysteine, catalyse by enzyme cystathionine-β-synthase with the help of essential cofactor pyridoxine (vitamin B6) [4]. Homocysteine act as an important biochemical intersection between methionine metabolism and biosynthesis of amino acid cysteine and taurine. Homocysteine present in plasma in various form -1% free as thiol, 70-80% bound to protein (albumin), 20-30% as homocysteine dimer or cysteine-homocystein disulfide bond. Several prospective studies have identified hyperhomocysteinemia as a strong and independent risk factor for atherosclerosis [5], although the pathological mechanism is still controversial. The prevalence of hyperhomocysteinemia in the Indian community have reported higher (incidence of 52 to 84%) as compare to west [6, 7, 8]. There are many factors like
genetic, dietary, disease and health condition, drugs predisposes person to hypercysteinemia. The present review describes the homocysteine metabolism, the association between homocysteine with incidence of cardiovascular diseases. Also the role of nutritional cofactors for decreasing the plasma homocysteine level and CVD risk are indicated. Finally, some recommendations are given for the nutritional therapy of patients with hyperhomocysteinemia. Further studies need to be performed to assess whether this will also reduce the risk of cognitive diseases and/or improve cognitive functioning.

2. Metabolism of Homocysteine

Homocysteine is a key determinant of the methylation cycle [9]. Homocysteine is a sulphur containing aminoacid, biosynthesize from methionine in several steps. Methionine undergoes S-adenosylation with condensation with ATP and forms S-adenosylmethionine (SAMe) [9]. S-adenosylmethionine is the principal methyl donor for all methylation reactions in cells. After donating its methyl group, SAMe becomes S-adenosylhomocysteine (SAH), which is then hydrolyzed into homocysteine and adenosine [10]. Homocysteine either remethylated to methionine via remethylation pathway or degraded to cystein via transsulfuration pathway (figure 1).

2.1. Remethylation

Homocysteine converted back to methionine via remethylation pathway, in which homocysteine methylated to methionine by taking on a methyl group provided, either by methylcobalamin, catalyzed by the enzyme methionine synthase (major pathway); or by trimethylglycine (betaine) catalyzed by the zinc-dependent enzyme betaine-homocysteine methyl -transferase (minor pathway). N5-methyltera-hydrofolate (active form of folate circulates in plasma) donates a methyl group to cobalamin, forming methylcobalamin, which remethylated homocysteine to methionine as shown in figure.1.

2.2. Transsulfuration

Excess of homocysteine converted to the sulphur amino acids cysteine via transsulfuration pathway, catalyzed by the enzyme cystathionine β-synthase, depended on nutritional cofactor pyridoxal 5'-phosphate (active vitamin B6) (Figure 1). Cysteine may also form glutathione, play important role in cellular defense, hence vitamin B6, B12, folate play important role in homocysteine- methionine metabolism.

3. Hyperhomocysteinemia

Hyperhomocysteinemia (HHcy) is characterized by an abnormally high level (above 15 μmol/L) of homocysteine (Hcy) in the blood. Various causes of hyperhomocysteinemia are shown in table 2. The main causes of hyperhomocysteinemia are vitamin deficiency (B6,B12,folate), genetic defects in enzyme (cystathionine-β-synthase deficiency and methylene tetrahydrofolate reductase), disease condition (renal failure, thyroid, diabetes dysfunction, psoriasis) interfere in the metabolism of vitamin B6, B12, folate. Plasma level of homocysteine is dependent on age, sex, menopausal condition, vegetarian diet and genetic background of the person. The lifestyle behaviors like smoking, coffee consumption, sedentary lifestyle causing plasma level of homocysteine [11]. The prevalence of hyperhomocysteinemia may vary significantly between populations. Incidence of hyperhomocysteinemia in Indian communities is much higher of 52 to 84% [12, 7] as compare to 5-7% in the American population [13, 14]. Men have higher fasting plasma Hcy than women [15], but this difference decreases with age and menopause. Postmenopausal woman have higher homocysteine value than premenopausal woman. With age homocysteine value increases, for elderly normal range of homocysteine is 5-20 μ mol/L [16,17], whereas for general population normal fasting total plasma Hcy range as 5 to 15 μ mol/l [18,19]. Based on homocysteine level in plasma, hyperhomocysteinemia may be classified as normal, mild, moderate and severe hyperhomocysteinemia as shown in table 1[20].Elevated Hcy is also a marker for vitamin B6, B12 and folate deficiencies. Hankey and Eikelboom 1999 [20] in his study found chronic renal
Table 1: Classification of Hyperhomocysteinemia

<table>
<thead>
<tr>
<th>Degree of hyperhomocysteinemia</th>
<th>Level of Homocysteine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>16-30 µmol/dl</td>
</tr>
<tr>
<td>Moderate</td>
<td>31-100 µmol/dl</td>
</tr>
<tr>
<td>Severe</td>
<td>&gt;100 µmol/dl</td>
</tr>
</tbody>
</table>

Table 2: Factor causing hyperhomocysteinemia

<table>
<thead>
<tr>
<th>Physiological determinants</th>
<th>Lifestyle factors</th>
<th>Diet</th>
<th>Genetic Causes</th>
<th>Diseases</th>
<th>Drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased age</td>
<td>Smoking</td>
<td>Low intake of vitamins, especially folic acid, vitamin B6, vitamin B12</td>
<td>Cystathionine β-Synthase deficiency</td>
<td>Chronic renal failure</td>
<td>Vitamin B12 antagonists (Nitrous oxide)</td>
</tr>
<tr>
<td>Male gender</td>
<td>High consumption of coffee</td>
<td>High intake of methionine-containing proteins</td>
<td>5MTHFR errors</td>
<td>Diabetes</td>
<td>Vitamin B6 antagonists</td>
</tr>
<tr>
<td>Menopause (HRT may lower homocysteine)</td>
<td>Alcohol consumption (moderate beer intake may be beneficial)</td>
<td>–</td>
<td>Methionine synthase deficiencies</td>
<td>Hypothyroidism</td>
<td>Estrogen-containing oral contraceptives</td>
</tr>
<tr>
<td>Increased muscle mass</td>
<td>Physical activity</td>
<td>–</td>
<td>Cobalamin mutations</td>
<td>Psoriasis</td>
<td>Metformin</td>
</tr>
<tr>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Malabsorption syndrome</td>
<td>Some antiepileptic drugs (phenobarbitol, valproate, phenytoin etc)</td>
</tr>
<tr>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Rheumatoid arthritis</td>
<td>Diuretic therapy</td>
</tr>
</tbody>
</table>

Disease patients have higher Hcy level than the moderately raised patients with atherothrombotic vascular disease, this is due to defective clearance of homocysteine by the kidney. This may contributes to the high incidence of vascular complications in chronic renal failure patients [20]. The levels of Hcy in plasma measured by employing techniques like ELISA test, immunoassays [21], enzymatic assays [22, 23] and HPLC [24]. Among them, HPLC and ELISA are more common.

4. Homocysteine and CVD

It is well evidence by several cross sectional and case control studies that moderate hyperhomocysteimia increases the risk factor atherosclerosis and CVD [25]. From table 3 we can see that the numbers of authors have found significant effect of homocysteine on CVD in their study. Cardiovascular diseases (CVD) comprises of diseases of heart and blood vessels. In the recent studies it was found that 10% risk of coronary artery diseases (CAD) attributed to hyperhomocysteinemia [26].Although possible mechanism explaining the relationship of plasma homocysteine level and CVD is inconclusive. The most possible hypothesis that elevated homocysteine levels is endothelium dysfunction due to enhanced oxidative stress and reduced the production and bioavailability of nitric oxide (a strong relaxing factor) of the endothelium [27]. There is growing evidence of an association between hyperhomocysteinemia and other health problems, including CVD, cognitive impairment, dementia, depression, osteoporotic fractures [28]. Homocysteine is an unstable amino acid produce free oxygen radicals [29].

Figure 2: Homocysteine and CVD

Figure 2 shows, hyperhomocysteinemia, thus causes increased production of free oxygen radicals, an oxidative stress and leads atherosclerosis. Atherosclerosis is the most common pathological condition that leads to CVD such as myocardial infarction, stroke, and heart failure [30]. This is believed to contribute to atherosclerosis in two ways. First is the free oxygen radicals oxidizes LDL (low density lipoprotein) to oxLDLc deposited in the sub-endothelial tissue. It acts as the key mediator of the inflammatory process in atherosclerosis. Macrophages take up oxLDLc get converted to foam cells. The foam cells get deposited below the endothelium to form a fatty streak, the first lesion in atherosclerosis. Other is the free oxygen radicals also combine with nitric oxide (NO), inactivating it...
Table 3: Contribution by the authors for effects of Hyperhomocysteine on CVD

<table>
<thead>
<tr>
<th>Sr.no.</th>
<th>Author</th>
<th>Study design</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ottar Nygård et al; 1995[31]</td>
<td>7591 men and 8585 women, 40 to 67 years of age</td>
<td>Elevated plasma Hcy level was associated with major components of the cardiovascular risk profile, ie, male sex, old age, smoking, high blood pressure, elevated cholesterol level, and lack of exercise.</td>
</tr>
<tr>
<td>2</td>
<td>Refsum et al, 1998[32]</td>
<td>Epidemiological studies of more than 10,000 patient</td>
<td>Elevated Hcy enhance the effect of the conventional risk factors, may be a strong predictor of cardiovascular mortality.</td>
</tr>
<tr>
<td>3</td>
<td>Arnesen et al; 1995, [33]</td>
<td>122CVD patient(M/F), 34-61yr(±51.3yr), Norway</td>
<td>Total serum homocysteine is an independent risk factor for CVD.</td>
</tr>
<tr>
<td>4</td>
<td>Nicholas et al; 1995[34]</td>
<td>229 men with IHD &amp; 1126 control, aged 35 to 64 years, London</td>
<td>Causal relationship between homocysteine level and IHD. Relationship seems stronger in younger persons than in older persons.</td>
</tr>
<tr>
<td>5</td>
<td>Folsom et al, 1998[35]</td>
<td>232 CHD patient, 45-64 yrs old</td>
<td>Finding was inconclusive</td>
</tr>
<tr>
<td>6</td>
<td>JAMA. 2002[36]</td>
<td>Meta-analysis of 5073 IHD events and 1113 stroke event</td>
<td>Elevated homocysteine is at most a modest independent predictor of IHD and stroke risk in healthy population</td>
</tr>
<tr>
<td>7</td>
<td>Selhub et al, 1995[37]</td>
<td>418 men and 623 women; age range, 67 to 96 years</td>
<td>High plasma homocysteine concentrations and low concentrations of folate and vitamin B6 are associated with an increased risk of extra cranial carotid-artery stenosis in the elderly.</td>
</tr>
<tr>
<td>8</td>
<td>Verhoef et al, 1996[38]</td>
<td>131 patient underwent coronary angiography and 101 control, aged 25 to 65 yrs, Netherlands.</td>
<td>Positive association between plasma Hcy and risk of severe coronary atherosclerosis</td>
</tr>
<tr>
<td>10</td>
<td>Perry, et al, 1995[40]</td>
<td>107 cases and 118 control men aged 40-59 years</td>
<td>Hcy is a strong and independent risk factor for stroke.</td>
</tr>
<tr>
<td>11</td>
<td>Venkata Madhav et al, 2013[41]</td>
<td>59 hyperhomocystemia and 6 control</td>
<td>Elevated serum homocysteine is a strong and modifiable risk factor of cerebral ischemic strokes</td>
</tr>
<tr>
<td>12</td>
<td>Boushey et al., 1995,[42]</td>
<td>Meta-analysis of 27 studies of about 4000 patients</td>
<td>Hcy was an independent, graded risk factor for atherosclerotic disease in the coronary, cerebral and peripheral vessels</td>
</tr>
<tr>
<td>13</td>
<td>Ashjazadeh N. et al, 2013[43]</td>
<td>171 ischemic stroke patients and 86 controls, aged over 16 years.</td>
<td>Elevated serum Hcy is an independent risk factor for ischemic stroke and it has a strong association with cardioembolic subtype.</td>
</tr>
<tr>
<td>14</td>
<td>Fallon et al; 2001[44]</td>
<td>107 men with ischemic stroke</td>
<td>No significant relation between homocyst(e)ine and ischaemic stroke</td>
</tr>
<tr>
<td>15</td>
<td>M Modi et.al; 2005[45]</td>
<td>Fifty-seven patients with ischemic stroke and 30 controls, Control, more than 30yrs of age.</td>
<td>Significant relation between homocysteine and ischaemic stroke. Strong positive correlation was also observed between hypertension, smoking, and high Hcy levels in the present study.</td>
</tr>
</tbody>
</table>
Nutritional cofactors (vitamins B6, B12 and folate) play an important role in the metabolism of methionine–Hcy cycle. Therefore deficiency of vitamin B (B6, B12 and folate) leads to impaired re-methylation of homocysteine to methionine and thus causes hyperhomocysteinemia [46]. Nutritional deficiency is one of the main reasons of hyperhomocysteinemia in Indian population. Majority of Indians are vegetarians so deficiency of vitamin B12 is quite common in them as non-vegetarian food is the main source of vitamin B12. Milk contains small amounts of vitamin B12 but most of it is destroyed by boiling. Vegetarians had a 4.4 times higher risk of low vitamin B12 than non - vegetarian and so a 3 times greater chance of hyperhomocysteinemia. Although main source of folic acid are vegetarian food, but deficiency of folic acid is also reported in Indian [47]. This may be due to longer periods of cooking, which can destroy upto 90% of the folic acid. Pyridoxine deficiency is also reported to be quite common amongst Indians. Deficiency of folate and vitaminB12 seems to have the greatest effect on homocysteine levels than vitaminB6. Many studies have shown folic acid supplementation has greater effect in reduction of Hcy level than other nutritional cofactors. According to a 1998 meta-analysis, folic acid supplementation (0.5-5 mg daily) results in a reduction of blood homocysteine of 25 percent [48]. Addition of 0.5 mg vitamin B12 reduces homocysteine concentrations another 7 percent. [49]. Vitamin B was shown to reduce homocysteine level, however its effect on improving endothelial dysfunction and lowering the risk of CVD is not yet proven [50].

6. Hyperhomocysteinemia – Treatment
Nutritional intervention, exercise and lifestyle modification is one of the efficient way to reducing the risk of cardiovascular diseases. In Indian community two-third of hyperhomocysteinemia is attributed due to nutrient deficiency. The best way to treat hyperhomocysteinemia is to incorporate Vitamin B rich food (B6, B12 and folic acid). The richest sources of these nutritional factors are given in table 4 whereas RDA and treatment recommendation are given in table 5. Many clinical trials have shown that supplementation of vitamin B (B6, B12, folic acid) greater than RDA lowers the plasma homocysteine level in CVD patients. Folic acid has greater effect in reducing homocysteine level than other B vitamin. Vitamin B12 and folic acid has profound effect on lowering fasting homocysteine whereas vitamin B6 lower homocysteine level after methionine loading. The most possible mechanism of folic acid supplementation in lowering homocysteine was to improve endothelial dysfunction in asymptomatic subjects with hyperhomocysteinemia [51] as well as in hyperhomocysteinemic patients. Besides nutritional intervention, physical activity, lifestyle modification like minimizing intake of tea, coffee, and smoking also lowers homocysteine level. Although different way to treat hyperhomocysteinemia are still controversial and need further studies and researches.

7. Conclusion
Previous researches have proven that moderate hyperhomocysteinemia increases the risk for cardiovascular disease. The most common causes of hyperhomocysteine in general population are related to genetic defects in enzymes or nutritional deficiency of vitamins involved in the homocysteine metabolism. Vitamin B6, B12 and folic acid are essential nutritional cofactors in homocysteine-methionine metabolism. The use of these nutritional cofactors found to be associated with decreasing plasma homocysteine level. However, many research works failed to demonstrate its beneficial effects in cardiovascular diseases. Recently there are a few very important papers published which favor folic acid in treatment, primary and secondary prevention of coronary artery disease. There need to be carried out new studies for uncovering novel nutritional strategies for lowering high homocysteine levels offering new possibilities for preventing cardiovascular disease.

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Folic acid</td>
<td>Green leafy vegetables (spinach, broccoli), legumes (lentil, chicken peas), orange</td>
</tr>
<tr>
<td>Vitamin B12</td>
<td>Beef, poultry, fish, soya, youghurt, egg yolk, fortified cereals, breads</td>
</tr>
<tr>
<td>Vitamin B6</td>
<td>Meat, poultry, fish, green leafy vegetables, legumes, potato, milk egg polk, grains wheat germ</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>RDA</th>
<th>Treatment Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Folic acid</td>
<td>400mcg</td>
<td>1mg</td>
</tr>
<tr>
<td>vitamin B6</td>
<td>2mg</td>
<td>10mg</td>
</tr>
<tr>
<td>Vitamin B12</td>
<td>6mcg</td>
<td>1mg</td>
</tr>
</tbody>
</table>

Table 4: Sources of Vitamin B (Nutritional cofactors)

Table 5: RDA and treatment recommendation of nutritional cofactors
References:


31. Ottar Nygard, MD; Stein Emil Vollset, MD, DrPH; Helga Refsum, MD; Inger Stensvold, MSc; Aage Tverdal, PhD; Jan Erik Nordrehaug, MD; Per Magne Ueland, MD; Gunnar Kvåle, MD Total Plasma Homocysteine and Cardiovascular Risk Profile: The Hordaland Homocysteine Study. JAMA. 1995; 274(19):1526-1533.


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