Biochemistry of homocysteine in health and diseases

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The amino acid homocysteine (Hcy), formed from methionine has profound importance in health and diseases. In normal circumstances, it is converted to cysteine and partly remethylated to methionine with the help of vit B12 and folate. However, when normal metabolism is disturbed, due to deficiency of cystathionine-β-synthase, which requires vit B6 for activation, Hcy is accumulated in the blood with an increase of methionine, resulting into mental retardation (homocystinuria type I). A decrease of cysteine may cause eye diseases, due to decrease in the synthesis of glutathione (antioxidant). In homocystinurias type II, III and IV, there is accumulation of Hcy, but a decrease of methionine, thus, there is no mental retardation. Homocysteinemia is found in Marfan syndrome, some cases of type I diabetes and is also linked to smoking and has genetic basis too. In hyperhomocysteinemias (HHcys), clinical manifestations are mental retardation and seizures (type I only), ectopia lentis, secondary glaucoma, optic atrophy, retinal detachment, skeletal abnormalities, osteoporosis, vascular changes, neurological dysfunction and psychiatric symptoms. Thrombotic and cardiovascular diseases may also be encountered. The harmful effects of homocysteinemias are due to (i) production of oxidants (reactive oxygen species) generated during oxidation of Hcy to homocysteine and disulphides in the blood. These could oxidize membrane lipids and proteins, (ii) Hcy can react with proteins with their thiols and form disulphides (thiolation), (iii) it can also be converted to highly reactive thiolactone which could react with the proteins forming -NH-CO- adducts, thus affecting the body proteins and enzymes. Homocystinuria type I is very rare (1 in 12 lakhs only) and is treated with supplementation of vit B6 and cystine. Others are more common and are treated with folate, vit B12 and in selected cases as in methionine synthase deficiency, methionine, avoiding excess. In this review, the role of elevated Hcy levels in cardiovascular, ocular, neurological and other diseases and the possible therapeutic measures, in addition to the molecular mechanisms involved in deleterious manifestations of homocysteinemia, have been discussed.

Keywords: Homocysteine metabolism, Homocystinurias, Homocysteinemias, Clinical manifestations, Thrombotic disorders, Cardiovascular diseases, Diabetes, Ocular diseases, Smokers, Oxidant stress, Protein thiolation, Protein homocysteinylfaction, Cystine, Vitamins B6, B12, folate.

Introduction

Homocysteine (Hcy) is one of the non-protein amino acids occurring in blood. It is a sulphur-containing amino acid with a free thiol (sulphydryl –SH) group and is formed from methionine through S-adenosyl methionine1.

Methionine → S-adenosyl methionine → homocysteine

It is easily oxidized in the blood to homocystine and disulphides, in which the thiol group is replaced by a disulphide (-S-S) group2.

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Abbreviations: Hcy, homocysteine; HHcys, hyperhomocysteinemias; CVD, cardiovascular disease; CHD, coronary heart disease; MTHFR, methylene tetrahydrofolate reductase.

It is converted to cysteine in the presence of an enzyme cystathionine-β-synthase and the reaction is irreversible3 (trans-sulphuration pathway).

Homocysteine → Cysteine

It can be converted back to methionine with vitamins, folate and vit B12 (a major reaction,
remethylation pathway) or choline via betaine (minor pathway)\(^1\) (Fig. 1). Thus, methionine, homocysteine, Hcy, cysteine and cystine are metabolically interrelated\(^4\).

Increased concentration of Hcy in the blood is called homocysteinemia. Because of oxidation of Hcy, urine in such cases contains homocystine referred to as homocystinuria. Thus, for abnormal metabolism of Hcy, the blood can be analyzed for Hcy or urine for homocystine or both. Blood total Hcy levels can also be estimated by reducing the disulphides.

Homocystinurias are a group of disorders associated with error in Hcy metabolism. Affected individuals excrete large amounts of homocystine in urine. Four types of homocystinurias referred to as types I, II, III and IV\(^5-7\) have been reported. Type I homocystinuria is the classical one and is due to the deficiency of cystathionine-\(\beta\)-synthase\(^7,8\) which requires pyridoxal phosphate (\(B_6\)) as coenzyme. The cystathionine-\(\beta\)-synthase catalyzes the trans-sulphuration pathway during the catabolism of methionine to cysteine (Fig. 2) and brings about the conversion of homocysteine to cystathionine. In case of a deficiency of this enzyme, as in the congenital homocystinuria, this step is blocked, resulting in an accumulation of Hcy and methionine, with a deficiency of the final product of the pathway i.e. cysteine\(^3,9\). Accumulation of methionine causes mental retardation, which is an important clinical evidence for type I homocystinuria. The prevalence of the type I homocystinuria is worldwide and in the ratio of 1:2,00,000.

Type II to IV homocystinurias are due to deficiency of the enzymes or absorption defects in the pathway of remethylation of Hcy to methionine\(^5,10\). For remethylation, the methyl group has to be supplied by \(N^5\)-methyltetrahydrofolate (\(N^5\)FH\(_4\)) through vitamin \(B_{12}\) as methyl \(B_{12}\). Hcy reacts with methyl \(B_{12}\) and is converted to methionine. So, both folate and vitamin \(B_{12}\) are necessary for remethylation. In type II homocystinuria, an enzyme \(N^5,N^{10}\)-methylene tetrahydrofolate reductase which acts on \(N^5,N^{10}\)-methylene tetrahydrofolate to give \(N^5\)-methyl \(FH_4\) is deficient. Thus, \(N^5\)-methyl \(FH_4\) is not formed\(^10\). This reaction is relevant, because adequate \(N^5\)-methyl \(FH_4\) is needed to remethylate Hcy through the formation of methyl \(B_{12}\). In type III, a methyl transferase apoenzyme (methionine synthase), which is required for the formation of methyl \(B_{12}\) from \(N^5\)-methyl \(FH_4\) and vitamin \(B_{12}\), is deficient\(^5,10\). Type IV homocystinuria is caused by defective absorption of vit \(B_{12}\) and lack of adequate methyl \(B_{12}\).

In type I homocystinuria, the enzyme is pyridoxal phosphate-dependent, so in the therapeutic aspect, pyridoxine or pyridoxal phosphate has to be considered to improve the efficiency of the available enzyme. In types II to IV, vitamin \(B_{12}\) and folate need to be advocated, as they are required for remethylation. In fact, unlike in type I, there is a deficiency of methionine in these types, hence there is no mental retardation and methionine supplementation may be helpful\(^3,10\) in selected cases, such as in case of methionine synthase deficiency. But, excess methionine may be avoided, as it would cause homocysteinemia. Thus, treatment for type I and other types is entirely different.

Kang and coworkers classified several types of hyperhomocysteinemias, in relation to total plasma Hcy concentration. They defined HHcy as severe, for concentrations higher than 100 µmol/L, intermediate for concentrations between 30 and 100 µmol/L, and
moderate for concentrations of 15 to 30 µmol/L, and a reference total plasma Hcy range as 5 to 15 µmol/l (mean, 10 µmol/L)\textsuperscript{2,11}.

**Clinical symptoms and manifestations**

The clinical symptoms of the type I homocystinuria are mental retardation, seizures, ectopia lentis (dislocation of eye lens), skeletal abnormalities including disproportionate growth, osteoporosis and vascular changes\textsuperscript{3}. Mental retardation in type I is the major symptom and vascular damage and atherothrombosis are the major causes of death in this type\textsuperscript{11,12}, as the heart is affected. The major symptoms in types II, III and IV vary with age and the central features are neurological dysfunction and psychiatric symptoms\textsuperscript{3}. Vascular lesions and thromboembolic diseases\textsuperscript{3} are also reported. Patients with defect in vitamin B\textsubscript{12} metabolism usually have psychomotor retardation, lethargy, megaloblastic anaemia and failure to thrive\textsuperscript{1,4}.

**Quantification**

Quantification of Hcy is important, as its values in plasma determine the pathological status. Elevated Hcy is also a marker for vitamin B\textsubscript{6}, B\textsubscript{12} and folate deficiencies. Hence, if a patient has elevated Hcy levels, with no systemic abnormality for the HHcys, then this increase could be attributed to low vitamin levels, either due to intake or absorption. Men have higher fasting plasma Hcy and post-methionine load Hcy than women\textsuperscript{5}. Also, most of the patients with elevated plasma Hcy, due to defects in cobalamin metabolism are reported to be very young\textsuperscript{3}. The homocystinuria in urine can be detected by the simple silver nitrate nitroprusside test\textsuperscript{13}. The levels of Hcy in plasma can be measured by employing techniques like ELISA (BioRad microplate EIA homocysteine kit), immunoassays\textsuperscript{14}, enzymatic assays\textsuperscript{15,16} and HPLC\textsuperscript{17,22}. Among them, HPLC and ELISA are more common, because of their reliability, sensitivity and reproducibility (Table 1).

**Homocysteine and vitamins**

In the metabolism of Hcy, water-soluble vitamins, vit B\textsubscript{6}, vit B\textsubscript{12} and folate play vital role as co-enzymes for the enzymes cystathionine-\(\beta\)-synthase, methyl transferase and methylene tetrahydrofolate reductase, respectively (Fig. 3). Hcy is also known as a sensitive functional marker of inadequate cellular folate and vit B\textsubscript{12} concentrations. Deficiency of these vitamins has important health consequences, in addition to their role in Hcy metabolism\textsuperscript{23}. Among persons of 12 to 39 yr of age, around 75% of the cases of high Hcy concentrations were associated with low folate or

<table>
<thead>
<tr>
<th>Reference range (µM)</th>
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<tr>
<td>Male</td>
<td>Female</td>
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<tr>
<td>10.8 ± 3.5*</td>
<td>3.5-9.9 (12-19 yr)</td>
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<tr>
<td>(35 yr)</td>
<td>5.9-15.3 (≥60 yr)</td>
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<td>4.9-11.6 (≥60 yr)</td>
<td>15.8 (range 7-23.7-adults, 16.5 (range 8.6-20.7-adults, 13-60 yr)</td>
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<td>13 to 60 yr)</td>
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*no separate data for sex

Fig. 3—Homocystinuria types II, III & IV (no mental retardation, therapy folate and B\textsubscript{12})
vit B₁₂ concentrations²⁴, suggesting the important role of vitamins in the Hcy metabolism²⁵. About 32% decrease in plasma Hcy levels was reported, when dietary food was supplemented with vit B₁₂ and folate²⁶. In aged people, interdependence of vitamins and Hcy was only about 30%, as metabolism slows down with age²⁴,²⁷. Another study also showed a strong association of increase in Hcy levels with age and nutritional status. Also, a strong inverse association was reported between plasma Hcy concentration and that of folate, vit B₁₂ and B₆. Individuals with low levels of these vitamins had high plasma homocysteine concentration²⁸. Thus, several studies have shown that supplementation of dietary folate and vit B₁₂ was efficient in bringing down the plasma Hcy levels.

**Homocysteine and diseases**

**Homocysteine and thrombotic diseases**

Hcy concentrations in patients with symptomatic vascular diseases are on an average 31% higher than in normal controls²⁹. The association between elevated Hcy levels and venous thrombosis was stronger among women than men³⁰,³¹. This might be partially caused by a more efficient trans-sulphuration in men, who present a lower response to the methionine overload than women of age-specific ranges³⁰. Many studies have shown association between HHcys and venous thrombotic episodes. However, in one of the studies no significant difference was found in plasma Hcy concentration of thromboembolism patients and control subjects³². While there are other risk markers for thrombosis like proteins C and S and antithrombin III deficiencies and activated protein C – resistance, HHcys has also become a risk factor for thrombosis³⁰. In one study, a higher rate of recurrent thrombosis was seen in patients with HHcys and with other defects like hypercholesterolemia³³ and non-insulin-dependent diabetes mellitus³⁴ than in patients without defects. Thus, there is evidence for the role of moderate HHcys in the development of premature and/or recurrent venous thromboembolic diseases.

**Homocysteine and cardiovascular disease (CVD)²,³⁰**

Hcy increases the tendency for blood to clot²,³⁰ often an immediate cause of a heart attack or stroke. An association between elevated plasma Hcy concentrations and increase in mortality due to coronary heart disease (CHD) in UK among Indian Asians, has been reported²². Hcy levels in Indian Asians were at least 6% higher than those of Europeans²². Raised Hcy concentration might be due to their reduced vit B₁₂ and folate levels and that consumption of more of dietary vitamins might reduce the risk for CHD²². CVD is reported as the major cause of death, both in the general population and patients with end stage renal disease (ESRD). Renal transplant recipients³⁵-³⁷, on recurrent CVD events possess a high prevalence of HHcys. By giving folate and vit B₁₂ to patients with chronic renal disease and thereby lowering the levels of Hcy could reduce the incidence of arteriosclerotic CVD effects³⁸.

**Homocysteine and type I diabetes**

Ischemic heart disease is the primary cause of death in diabetes, especially at younger age of illness. Hcy has emerged as one of the risk factors for the development of atherosclerotic changes in diabetes. In a study conducted in children with diabetes for duration of 3-5 yr and normal healthy children³⁹, elevated levels of Hcy were observed in children with longer duration of illness, when compared to the control group, although the results were not statistically significant. Elevated Hcy levels in the prolonged type I diabetic children indicated that they were particularly exposed to some atherosclerotic changes, independent of the conventional metabolic control and other lipid metabolism-involved pathways. This shows that the reduction of blood levels of Hcy may also be one of the strategies for the treatment of diabetes.

**Homocysteine and ocular complications**

In patients untreated with vitamins and amino acids, ocular complications include ectopia lentis, secondary glaucoma, optic atrophy and retinal detachment⁴⁰. There are no characteristic signs or symptoms of ocular complications in infancy and Hcy levels of new born babies⁴¹,⁴². However, Hcy levels of newborn babies may be used for detection of such complications. In addition to above symptoms, cataract is also reported sometimes. In a recent study, we found association of homocystinuria with congenital/developmental cataract in children. Nutritional deficiency, especially of vitamins may be responsible for the high Hcy levels in these children and the cataract in them may be due to the oxidative stress caused by Hcy¹⁰. The association of homocysteinemia with retinal occlusions has also been reported. In a recent study, elevated Hcy level has been found to be an independent risk factor for retinal vascular occlusive disease⁴³. This risk is high
for retinal arterial disease and central retinal vein occlusive disease, compared to its role in some other eye diseases\(^{44,45}\). Thus, in addition to conventional cardiovascular risk factors, measurement of total homocysteine (tHcy) may be important in the initial investigation and management of retinal vascular occlusive disease. Lowering of Hcy levels by administration of folate and vit B\(_{12}\) may be helpful in the prognosis of patients with such disease\(^{46}\). In another study, HHcy was reported to be prevalent in patients with arterial occlusion\(^{47}\). In patients with non-aortic ischemic optic neuropathy (NAION), incidence of hypertension and ischemic heart disease was significantly high with HHcys\(^{48}\).

**Homocysteine and Marfan syndrome**

It is difficult to diagnose the two disorders as both have similar ocular abnormalities\(^{49}\) and other similar clinical manifestations, such as skeletal deformabilities, cardiovascular problems and generalized osteoporosis. It is likely that there might be a common biochemical cause for both the diseases. This may be linked to the collagen abnormality, as HHcy has been reported to have effect on the copper-containing lysyl oxidase\(^{50}\), a major enzyme involved in the collagen modification. There might also be defect in the metabolism of copper\(^{51}\), which is a cofactor required for the enzyme activity. Thus, further studies are needed to trace out the link between the two disorders.

**Homocysteine and smoking**

Smoking is a known risk factor for atherosclerotic disease\(^{52}\). Its involvement in atherosclerotic vascular disease (ASVD) might be due to the redox changes in glutathione\(^{52}\). In a pilot study, conducted in healthy young subjects, differing in their smoking habits, a positive statistical difference was observed in the level of plasma Hcy (-SH) between smokers and non-smokers, compared to the other related amino thiols\(^{51}\). More than a two-fold increase in Hcy level in smokers was related to the extent of oxidation by oxygen free radicals or ONO\(_2\) and their complex actions, taking place in cigarette smoking. Sulphydryl groups in thiols have an important role in scavenging free radicals that can be formed in smoking\(^{53}\).

**Homocysteine and genetics**

Homocystinurias are inherited as autosomal recessive disorder. The defect may be due to a genetic defect in the gene of cystathionine-\(\beta\)-synthase\(^3\) or methylene tetrahydrofolate reductase (MTHFR). A thermolabile variant of MTHFR could be formed due to genetic disorder\(^3\). Hyperhomocysteinemia was observed a patient taking antifolate drug for a long time for psoriasis and also in a patient with malabsorption syndrome. These were due to secondary deficiency of folate/vit B\(_{12}\) reflecting as acquired homocysteinemia\(^54\).

**Molecular mechanisms of homocysteine**

The various deleterious manifestations of homocysteinemia are due to oxidant stress, protein thiolation and protein homocysteinylilation.

**Oxidant stress**

Hcy exerts its effects through a mechanism involving oxidative damage\(^{55,56}\). In the plasma, it continuously undergoes oxidation, leading to the formation of homocysteine, Hcy-mixed disulphides and thiolyated proteins\(^{57,58}\). During oxidation of sulphhydryl groups of Hcy, and other –SH–containing compounds like cysteine, reactive oxygen species namely superoxide anion, hydrogen peroxide and hydroxyl free radicals are generated, resulting in the endothelial cytotoxicity by oxidizing membrane lipids and proteins\(^59\). Hcy also attenuates endothelial availability of nitric oxide, which is removed as peroxynitrite\(^60,61\). Nitric oxide in physiological amounts is needed for desirable vasorelaxation and inhibits platelet adhesion\(^62,63\).

\[
\text{Homocysteine} \xrightarrow{\text{oxygen}} \text{Homocysteine + Reactive oxygen species}.
\]

(Superoxide anion, H\(_2\)O\(_2\) and OH\(^-\))

**Protein thiolation**

Free Hcy reacts non-enzymatically\(^57\) with the sulphydryl residues of the body proteins forming adducts with disulphide linkages (Fig. 4). The reaction is called thiolation. About 70% of Hcy in the blood is thiolated\(^3\). The thiolation depends on the levels of Hcy in the blood; the higher the Hcy levels, the higher will be the thiolation\(^54\). Extensive thiolation has been found to affect the function of proteins and enzymes\(^64\).

**Protein homocysteinylilation**

The harmful effects of Hcy may also be due to the formation of Hcy thiolactone, a cyclic thioester, formed as a metabolite of Hcy, when the normal
trans-sulphuration or remethylation pathways are affected. In vivo, there could be error-editing by methionine tRNA synthase, which reacts with Hcy instead of methionine, resulting in the formation of tRNA-Hcy-AMP complex which is converted Hcy thiolactone.

\[
tRNA \text{ methionine synthase-Hcys} + \text{ATP} \rightarrow \text{tRNA–homocysteine–AMP}
\]

\[
\text{tRNA–homocysteine–AMP} \rightarrow \text{homocysteine–AMP}
\]

\[
\text{homocysteine–AMP} \rightarrow \text{homocysteine thiolactone}
\]

Hcy thiolactone is highly reactive and easily acylates free amino groups of proteins under physiological conditions. It reacts with protein-lysine at the epsilon amino group forming a –NH–CO– adduct. This adduct formation could be with many available lysine amino residues. The process is called homocysteinylaton (Fig. 5). Conditions, such as elevated plasma Hcy levels favour its production and hence result in the concomitant increase in the degree of protein homocysteinylaton. Homocysteinylaton of 33% and 88% of lysine residues in methionine synthase and trypsin, respectively, resulted in complete loss of their enzymatic activities. Homocysteinylaton may also lead to protein functional damage by other mechanisms like inactivation of the important enzyme lysyl oxidase, which is required for collagen modification through derivatization of the active site of a tyrosine quinone cofactor. Homocysteinylated proteins are prone to multimerization and structural changes, which lead to their denaturation. In addition, homocysteinylaton could also generate modified proteins like haemoglobin, LDL and plasma proteins that are physiologically detrimental. In addition to homocysteinylaton, Hcy thiolactone also thiolates the –SH groups of the same proteins (Fig. 5).

The vascular damage, a major condition associated with elevated plasma Hcy levels may be due to protein homocysteinylaton. The homocysteinylated proteins accumulate in the vascular wall surfaces (Fig. 6), which are recognized by the macrophages and then phagocytosis of the cells occurs, resulting in the destruction of the endothelial cells. Alternately, homocysteinylated proteins on vascular cell surface could be recognized by anti-Hcy antibodies, with the formation of antigen-antibody immune complexes on the surface of the vessels. These complexes are recognized and phagocytosed by the macrophages and
hence the endothelial cells might be destroyed. If the agent responsible for the injury (homocysteinylated proteins) is present continuously, attempts to repair the damaged vascular wall would eventually lead to an atherosclerotic plaque\textsuperscript{2,65}. Recently, homocysteinemia in uveitis\textsuperscript{68} and age-related macular degeneration\textsuperscript{69} and homocysteinylation of plasma proteins in uveitis\textsuperscript{70} and Eales’ disease\textsuperscript{71} have also been reported.

**Possible treatment for homocysteinemia**

Treatment of homocysteinemia at the early stage of diagnosis is very essential, as it helps in prevention of further complications of HHcy. For the prevention and treatment, the type of homocystinuria-homocysteinemia, existing in the patients i.e., type I or other types must be known. Patients with cataract or ectopia lentis and homocystinuria are usually treated surgically by lensectomy. For type I homocystinuria, the recommended treatment usually is a methionine-low diet and intake of cystine supplement. If the patients are vit B\textsubscript{6} responsive, intake of vit B\textsubscript{6} is beneficial. The treatment with the supplementation of vit B\textsubscript{12} and folate is beneficial in case of patients with types II, III or IV homocystinurias, if they possess only nutritional deficiency of the vitamins. Betaine and/or methionine supplementation is also useful in these types\textsuperscript{30}, if there is a deficiency of functional methionine synthase or thermostable MTHFR\textsuperscript{30}. The levels of methionine have to be monitored, as an excess of methionine could itself cause homocysteinemia\textsuperscript{3,30}. In addition, due to oxidant stress, antioxidant GSH was found to be less in patients with homocystinuria of all the types. So, treating these patients with GSH might be helpful in building up antioxidant defense in all the types\textsuperscript{10,53}.

**Conclusion**

Homocystinurias of types I, II, III and IV/homocysteinemias have been discussed, in relation to their role in different diseases, namely cardiovascular, thrombotic, diabetes, Marfan, ocular diseases and in smoking, and the molecular mechanisms and genetics. Their deleterious effects could be due to protein thiolation, protein homocysteinylation and through reactive oxygen species, generated on the oxidation of Hcy. Depending upon whether the defect is in trans sulphuration or remethylation pathway, suitable therapy has to be instituted. For type I homocystinuria (cystathionine-β-synthase deficiency) with mental retardation, supplementation with vit B\textsubscript{6}, cystine with methionine-low diet is helpful. For the other types, it is mainly the supplementation of folate and, if desired vit B\textsubscript{12} and in selected cases, such as in functional deficiency of methionine synthase or methylene terahydrofolate reductase, betaine or methionine could be given, but monitoring of the level of methionine is essential.

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