HYPERHOMOCYSTEINEMIA: ITS IMPACT ON CARDIOVASCULAR DISEASE AND ATHEROSCLEROSIS

Israa A. Jalal[*], Abeer Elkhoely, Shimaa K. Mohamed and Amany A.E. Ahmed

Department of Pharmacology and Toxicology, Faculty of Pharmacy - Helwan University, Cairo, Egypt

Hyperhomocysteinemia (HHcy) was found to be a risk factor for cardiovascular disorders and many other diseases. HHcy occurs when homocysteine (Hcy) exceeds 15 μmol/L. The prevalence of HHcy ranges from 5 – 10% in the general adult population. HHcy can cause atherosclerosis and heart dysfunction through oxidative stress, inflammation, endothelial interaction with leukocytes, coagulation, endothelial fibrosis, SMCs proliferation, apoptosis, lipids dysregulation, epigenetic modifications and modifications of electrical conduction in the heart. Some of the drugs that have been studied have been shown to be effective in reducing the level of HHcy. Conclusion: High Hcy level can be a strong cause in several disorders including cardiovascular diseases. So, serious effort should be made to avoid and treat HHcy. All evidence suggests that Hcy is causally connected to atherosclerosis by many mechanisms. But future studies should concentrate on the molecular pathways of HHcy in cardiac dysfunction, and the effects of Hcy-lowering medications on cardiac dysfunction.

Keywords: Homocysteine, hyperhomocysteinemia, cardiac dysfunction, atherosclerosis, ER stress and oxidative stress

INTRODUCTION

Cardiovascular diseases are the major cause of death in both developing and developed countries[1]. Hyperhomocysteinemia (HHcy) is a risk factor for atherosclerosis and cardiovascular diseases. Atherosclerosis is a chronic inflammatory condition in which cholesterol, fatty deposits, calcium, cellular waste products, and other chemicals accumulate in the endothelium layer, narrowing the arteries[2]. HHcy, which is a medical condition characterized by an abnormal concentration of homocysteine (Hcy, a sulfur-containing non-protein amino acid that is found in blood plasma normally) in the blood, has been found to present in a wide range of diseases[3].

Hyperhomocysteinemia

Normal plasma Hcy range is (5–15 μmol/L). HHcy occurs when there is a presence of an inborn error of homocysteine metabolism. Moderate, intermediate, and severe HHcy are defined as basal values for plasma homocysteine less than 30, between 31 and 100, and above 100 μmol / L, respectively[4].

Epidemiology

The prevalence of HHcy ranges from 5 – 10% in the general adult population, with maxima of 30% among the geriatric[5]. Findings from large-scale longitudinal and observational research indicates that those with HHcy and high blood pressure have a greater risk of stroke than those who do not have either condition[6].

Biosynthesis and metabolism of Homocysteine

Hcy in humans, is generated from Methionine (MET) containing diets, MET is converted into cofactor: S- adenosylmethionine (SAM; a universal methyl donor.) In a controllable way, the reaction's result is adenosylhomocysteine (SAH) which is

*Corresponding author: Israa A. Jalal, E-mail: Israa.AdelJalal@pharm.helwan.edu.eg

converted to Hcy. Then, Hcy is metabolized by three pathways: the remethylation pathway (folic acid and vitamin B12 dependent), in which Hcy is reconverted to MET, and the transsulfuration pathway (vitamin B6 dependent), in which Hcy is transformed to cystathionine then cysteine, or cyclized to generate homocysteine thiolactone (HTL)[7] **(Fig.1).**

Although Hcy is produced in all human organs, it is mostly detoxified in the liver and kidney. Re-methylation is the only route to detoxify Hcy in human skin and vascular tissues; as, transsulfuration pathway enzymes are not produced in such tissues[7].

The first step is the interaction between MET and ATP producing SAM (AdoMET). After transferring the methyl group to acceptor molecules (DNA, RNA, proteins, etc.), SAH is produced. Then, Adenosyl homocysteinase transforms SAH into Hcy. The methyl group from 5-N-methyl tetrahydrofolate can be used to remethylate the

Hcy back to MET by MET synthase (MS). Whereas 5-N-methyl tetrahydrofolate is the most common methyl donor in the remethylation pathway, choline and betaine can also be used. However, the betaine route, which is driven by betaine-Hcy-methyl-transferase, is primarily limited to the liver. Hcy can also generate cystathionine by combining with serine. Cystathionine-β-synthase (CβS) catalyzes this reaction[8].

Furthermore, Hcy can be cyclized to create HTL, which is regarded as the hazardous product of Hcy. An error-editing reaction in protein biosynthesis is thought to be responsible for the creation of HTL. Because MET and Hcy are structurally similar, methionyl-tRNA synthetase prefers to use Hcy over Met. The AMP is lost from the activated Hcy or adenylated Hcy (not S-adenosyl-Hcy) resulting in the cyclization process and formation of HTL, although this error is promptly corrected[8].
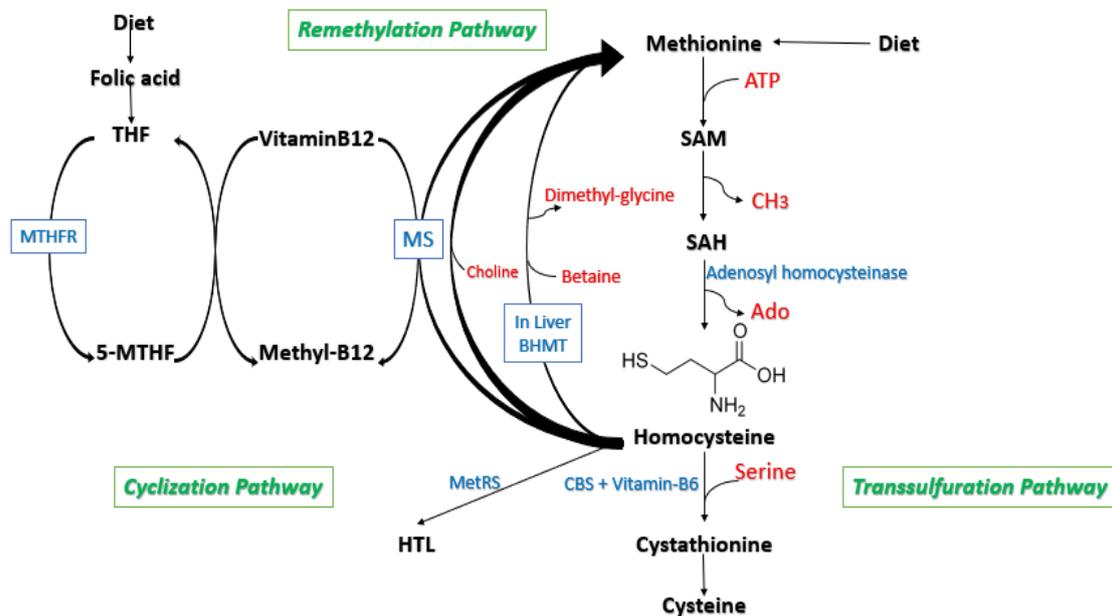
Fig. 1: *Biosynthesis and metabolism of Homocysteine (Hcy): first, dietary Methionine interacts with ATP producing S-Adenosyl-L-methionine (SAM). After transferring the methyl group to acceptor molecules, S-Adenosyl homocysteine (SAH) is produced. Then, Adenosyl homocysteinase transforms SAH into Hcy. The methyl group from 5-N-methyl tetrahydrofolate can be used to remethylate the Hcy back to MET (MET synthase (MS); vitamin B12-dependent) (remethylation pathway). choline and betaine can also be used for remethylation However, the betaine route, which is driven by betaine-Hcy-methyl-transferase, is primarily limited to the liver. Hcy can also generate cystathionine by combining it with serine (Cystathionine-β-synthase (CβS; vitamin B6-dependent activity) (transsulfuration pathway). MTHFR, Methylenetetrahydrofolate reductase.*

Etiology

Genetic- induced

Disease probabilities are linked to genetic variations in important enzymes such as MS, MTRR, MTHFR, and CBS[9] (**Fig.2**).

CBS deficiency

Because CBS is an enzyme that participates in the transsulfuration pathway, which converts Hcy to cystathionine, a defect in CBS is the most prevalent cause of HHcy. Human CBS is found in the brain, liver, muscle, kidney, and ovaries, as well as in the nerves and heart during early embryonic development. An inadequate amount of Hcy is converted to cystathionine when CBS malfunctioned. The transsulfuration pathway does not convert any Hcy to cystathionine in the lack of CBS. In several ethnic groups, a T833C polymorphism in CBS has been identified to produce mild HHcy. Moreover, not every CBS polymorphism causes HHcy[10].

MTHFR deficiency

A mutation in the gene coding for the enzyme (MTHFR) causes moderate elevation of Hcy [11]. C677T polymorphism is one of the most studied polymorphisms. According to estimates, 10% of the global population is homozygous for the common C677T polymorphism, even though the actual causes of high C677T polymorphism incidence in specific parts of the world are still being investigated, knowing the prevalence of these polymorphisms in different parts of the world could be useful for therapeutic treatment. Indeed, the strong association between MTHFR polymorphisms and levels of folate in the mothers' serum expressed concern about the use of folic acid- containing dietary supplements by pregnant[12].

MSR deficiency

MS (CblG) and methionine synthase reductase (MSR) (CblE) deficiency, as well as abnormalities of intracellular cobalamin metabolism that decrease MS function and produce homocystinuria, are rare illnesses[13]. MS activity is dependent on reactivation, which is performed by a reductase (MSR) converting cobalamin II to cobalamin I[14]. CblG is caused by mutations in the MTR gene, which codes for MS, resulting in aberrant enzyme action[15].

Nutritional- induced

Nutritional inadequacies of several of the cofactors implicated in Hcy metabolism (**Fig.2**).

B vitamin deficient (folate, B6, B12) diets

The absorption of the three dietary vitamins, vitamin B12, folic acid, and vitamin B6, is required for normal Hcy metabolism. Folic acid, also known as pteroylmonoglutamic acid, is a precursor of 5- methyl-THF, which is essential for optimal MS activity. Folates contribute to the synthesis of important molecules (like purines) by boosting SAM levels[10].

Folic acid intake of 0.5– 5.0 mg/ day has a larger effect on decreasing plasmatic Hcy levels than other cofactors. vitamin B12 and folic acid co-administration has a synergistic effect on Hcy levels. Because vitamin B6 cannot be produced in the body, it is easily detectable, and its blood level is low in all ages. Only a serious and long-term vitamin B6 deficiency might influence basal Hcy[4].

However, these vitamins are easily lost through urination since they are water-soluble. In elders, a loss of vitamin B12, cobalamin and vitamin B is frequent. As a result, Hcy levels rise as people get older. Vitamin B12 deficiency is rare in adults who eat a balanced diet, as overall body reserve can surpass 2500 mcg and daily cycling is modest[10].

Cobalamin (an organometallic molecule that is essential for the normal action of MS) unlike folic acid, is only present in animal meats or dairy products, putting vegans at danger of shortage[16].

The cofactor pyridoxine phosphate is required for normal CBS enzyme function. Pyridoxine phosphate is available in all kinds of foods and is stored in the liver, therefore nutritional shortage is unusual. The risk of pyridoxine deficit is higher in patients with a combined liver illness and low nutritional condition, such as alcoholism (1.5 g of alcohol/ kg/ d, >5 years)[17].

High Methionine diet

The only source of Hcy accessible in diet is MET, a necessary amino acid in animals. The development of HHcy is induced by a high- MET diet e.g. turkey, fish, milk and Tofu. Urinary HTL concentrations were substantially greater in mice fed a high- MET or high- Hcy diet than in mice provided a regular, balanced diet. The high- MET diet altered blood HTL levels as well, but the difference was insignificant[10].

Alcohol- induced

As previously stated, persistent alcohol consumption raises the likelihood of pyridoxine deficiency (Fifure2). It also interacts with folic acid and cyanocobalamin metabolism. Furthermore, it is linked to gastrointestinal problems, which cause a reduction in vitamin and folic acid absorption, contributing to high Hcy levels[18].

Drug- induced

HHcy can be caused by the overuse of certain medicines **(Figre2, Table1).**

Renal dysfunction

The mechanism through which chronic renal failure enhances Hcy levels is uncertain **(Fig.2).** Reduced renal clearance of Hcy and non-renal disposition of drugs are two hypothesized pathways. Increased Hcy levels are linked to a lower glomerular filtration rate (GFR) in theories that indicate slower elimination as the primary cause. The typical GFR is 100 mL/ min, while a GFR of 60 mL/ min indicates a modest decrease of function in the kidney. HHcy, on the other hand, occurs at a GFR of roughly 60 mL/ min, and the likelihood of HHcy is 85– 100% in end-stage renal disease (ESRD)[10].

Despite the lack of a clear mechanism, a negative linear correlation has been found between Hcy levels and GFR. Furthermore, renal failure appears to have a role in the inhibition of key enzymes involved in Hcy metabolism. Human kidney tissue contains Hcy remethylation and transsulfuration enzymes, which are inactivated in chronic renal failure[10].

HHcy is not caused by the kidney's inability to eliminate Hcy. Patients with renal failure exhibit aberrant folate metabolism and elevated AdoMet and AdoHcy levels. These are nonrenal variables that can cause HHcy by inhibiting the remethylation of Hcy and cysteine excretion, respectively[10]. On the other hand, renal failure is thought to produce the accumulation of uremic toxins, which then disrupt Hcy metabolism, resulting in HHcy[28].

Others

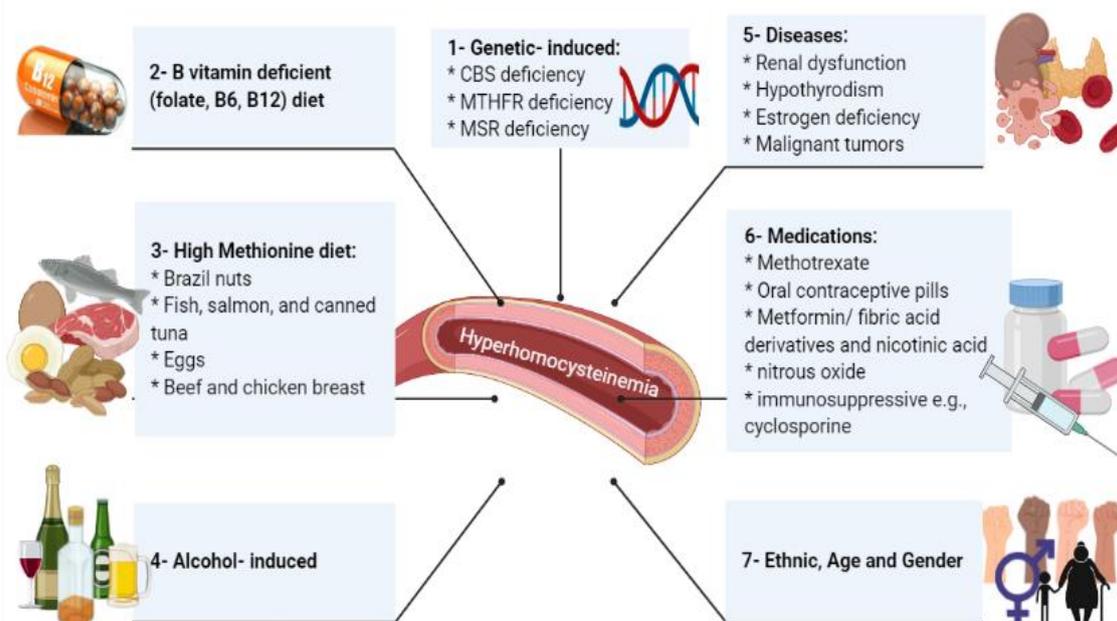Other diseases can participate in HHcy, including[10] **(Fig.2):**

Fig. 2: *Etiology of Hyperhomocysteinemia, Created with BioRender.com.*

Table 1: Some drugs- induced Hyperhomocysteinemia.

Drug	Mechanism	Ref.
1. L-DOPA	L-DOPA is metabolized by Catechol-O-methyl transferase (COMT) leading to increase SAH levels	19
2. Trimethoprim	It prevents remethylation by blocking dihydrofolate-reductase (DHFR), which transforms dihydrofolate to active tetrahydrofolate.	20
3. H2- receptor antagonists and/or PPIs	It reduces the absorption of protein- bound vitamin B12 in the diet.	21
4. Metformin	It interferes with vitamin B12 absorption.	22
5. Theophylline, isoniazid, and hydralazine	Inhibitor of vitamin B6 (phosphodiesterase inhibitor of pyridoxal phosphate).	23
6. Nitrous oxide	Vitamin B12 cofactor inactivator; inhibits methionine synthase.	24
7. Anticonvulsants— carbamazepine, phenobarbital, phenytoin, primidone, valproic acid	It disrupts folate metabolism by reducing intestinal absorption, activating hepatic enzymes which need and deplete folate, and messing with folate coenzyme metabolism.	23
8. 6-Azauridine	Inhibitor of vitamin B6.	23
9. Cyclosporine	Possibly acts as folate antagonist; preventing homocysteine remethylation with folate.	23
10. Methotrexate	Blocks the folate cycle by inhibiting dihydrofolate reductase and lowering 5-methyltetrahydrofolate levels.	23
11. Loop diuretics: Furosemide/ Torsemide	It raises the rate of vitamin B6 and folic acid excretion.	25
12. Cholestyramine	It hinders the absorption of folate and vitamin B12.	26
13. Nicotinic acid	It inhibits pyridoxal kinase, resulting in lower vitamin B6 levels and CBS activity.	26
14. Fibric acid derivatives (e.g., fenofibrate)	Unknown mechanism	27

Cardiovascular Effects of Hyperhomocysteinemia:

    a. Pathways of Hyperhomocysteinemia- induced atherosclerosis **(Fig.3)**:

The mechanisms of how Hcy induces atherosclerosis could be explained as the follow:

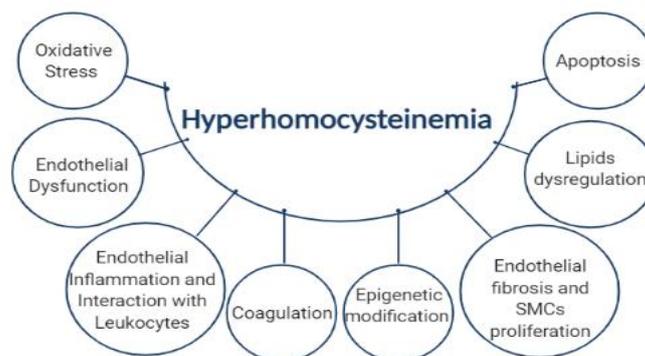**Homocysteine- Induced Oxidative Stress and Endothelial Dysfunction**

Hcy's highly reactive thiol group undergoes an autoxidation reaction with a cation like copper, resulting in production of superoxide anion, hydrogen peroxide, and hydroxyl radical both vascular cells and circulating leukocytes[29] **(Fig.4).**

On the other hand, Hcy may cause oxidative stress indirectly by decreasing the expression and activities of antioxidants thioredoxin, superoxide dismutase (SOD), glutathione peroxidase (PGX-1) and heme oxygenase 1 (HO-1), all of which have been linked to oxidative stress and endothelial damage[30] **(Fig.4).**

Superoxide anion interacts with NO decreasing it and creating peroxynitrite (ONOO-), both induce lipid peroxidation of cell membranes[31]. Furthermore, High amounts of ROS lead to suppression of dimethylarginine dimethylaminohydrolase (DDAH, an enzyme involved in the metabolism of asymmetric dimethylarginine ADMA which is an endogenous inhibitor of NO synthase by competing with L-arginine) which in turn has been found to impede NO production[32] **(Fig.4).**

The activity of the L-arginine transporter CAT-1 in endothelial cells is likewise reduced by Hcy-induced ROS which in turn leads to endothelial NOS (eNOS) uncoupling, eventually reduces NO synthesis and increases ROS generation[33] **(Fig.4).**

Fig. 3: *Pathways of Hyperhomocysteinemia- induced atherosclerosis.*
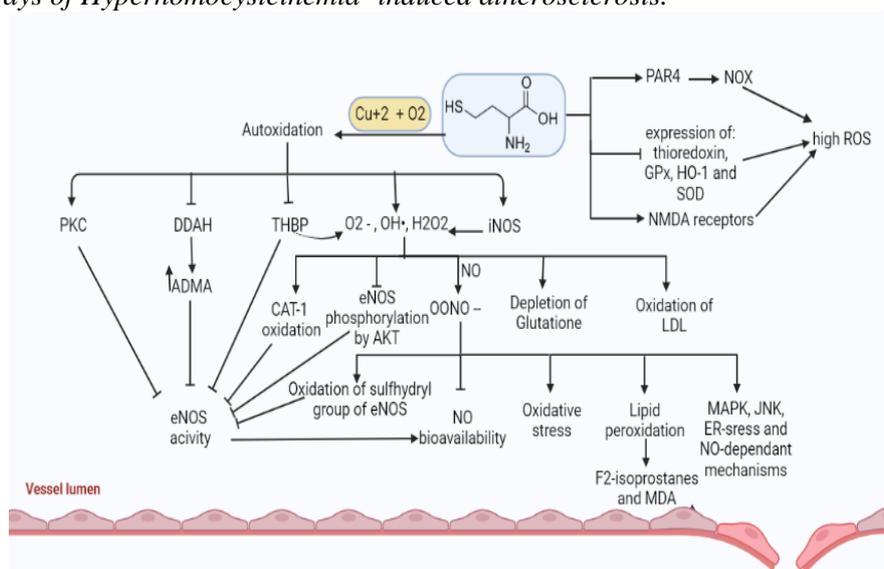
Fig. 4 : Homocysteine (Hcy)- Induced Oxidative Stress and Endothelial Dysfunction: Cu+2;copper, O2-; superoxide anion, H2O2; hydrogen peroxide, OH•; hydroxyl radical, NO; nitric oxide, SOD; superoxide dismutase, GPx; glutathione peroxidase, HO-1; heme oxygenase 1, NMDA; N-methyl-D-aspartate, ROS; reactive oxygen species, OONO-; peroxynitrite, THBP; tetrahydrobiopterin, DDAH; dimethylarginine dimethylaminohydrolase, ADMA; asymmetric dimethylarginine, CAT-1; Cationic Amino Acid Transporter-1, eNOS; endothelial NOS, LDL; low-density lipoproteins, MDA; malondialdehyde, MAPK; mitogen-activated protein kinase, JNK; c-Jun N-terminal kinase. Created with BioRender.com

The related thiolactone (HTL), which is created by an error- editing action of aminoacyl- tRNA synthetases with Hcy and ATP, is primarily responsible for N-homocysteinylation. While S-homocysteinylation happens when Hcy forms a disulphide bond with a protein by binding its free thiol group to another free thiol group of a cysteine residue in the protein molecule. Homocysteinylated eNOS inhibits NOS activity[34], while homocysteinylated metallothionein promotes ROS buildup in ECs[35]. Homocysteinylated ACE, on the other hand, has a higher potential to generate Ang II in ECs and hence activates the Ang II– NOX– ROS pathway[36].

Furthermore, by activating protease-activated receptor-4, which upregulates NOX, HHcy produces dose-dependent damage to heart microvascular endothelial cells [37].

By interrupting disulphide bond formation and causing misfolding of proteins traversing the ER, Hcy oxidative stress causes ER stress[38] **(Fig.4)**. Increased intracellular Hcy levels may boost the expression of ER stress response genes like GRP-78, GRP-94, RTP and Herp [39]. Furthermore, ER stress is linked to the release of ER Ca2+ reserves, which can contribute to

oxidative stress via mitochondrial effects and NF-κB activation, resulting in inflammation[40].

Glutamate receptors AMPA and NMDA, have been identified as possible Hcy cell surface receptors **(Fig.4)**. In neutrophils, monocytes/macrophages, lymphocytes, ECs and VSMCs, NMDA receptors are expressed. NMDA receptor antagonist prevented oxidative damage caused by Hcy, Hcy- induced proliferation and mitochondrial toxicity in ECs. Furthermore, Hcy increased expression of COX-2 by ROS produced by NMDA- mediated calcium signaling pathways in murine macrophages, which are inhibited by NMDA antagonist[41].

Homocysteine Promotes Endothelial Inflammation and Interaction with Leukocytes

The highly regulated process of inflammation is thought to have a major role in the development and progression of atherosclerosis. The proinflammatory action of HHcy is linked to the production of ROS and the activation of nuclear transcription factor B (NF-κB), ERK1/2, as well as PKC and calmodulin, which regulate adhesion molecules, chemokines and cytokines **(Fig.5).** NF-κB is present in the cytosol in an inactive form under normal physiological conditions. Stimuli like homocysteine and reactive oxygen species (ROS) phosphorylate the inhibitory protein IκB alpha, causing it to be degraded, and NF-κB to be translocated to the nucleus, where the target genes are activated. The expression of intercellular adhesion molecule-1 (ICAM-1), monocyte chemoattractant protein-1 (MCP-1/ CCL2), vascular adhesion molecule-1 (VCAM-1), soluble cell adhesion molecule-1 (sCAM-1) and E and P-selectins has been shown to increase following Hcy-induced NF-κB activation[42-45].
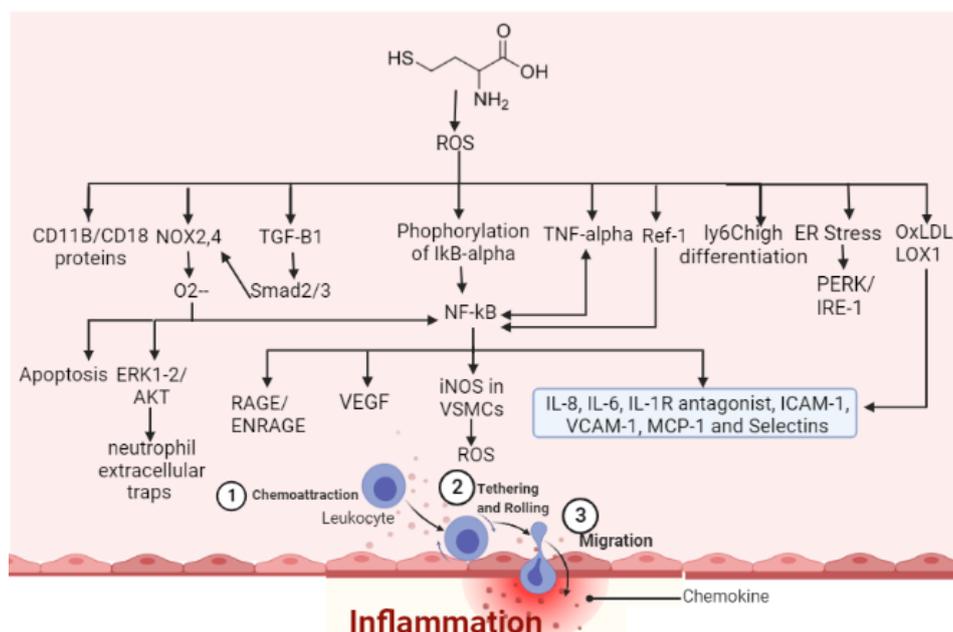
Fig. 5: Homocysteine Promotes Endothelial Inflammation and Interaction with Leukocytes: NF-kB; nuclear transcription factor kappa B, ERK1/2; extracellular signal-regulated kinases 1 and 2, TNF-alpha; tumor necrosis factor alpha, NOX2,4; NADPH oxidase2 and 4, TGF-B1; transforming growth factor beta1, Ref-1; redox factor-1, ER; endoplasmic reticulum, oxLDL; oxidized LDL, LOX1; lectin-type oxidized LDL receptor 1, O2-; superoxide anion, PERK; Protein kinase R-like ER kinase, IRE-1; Inositol-requiring enzyme 1, VSMCs; vascular smooth muscle cells RAGE; receptor for advanced glycation end products, EN-RAGE; ligand of RAGE, VEGF; Vascular endothelial growth factor, ICAM-1; intercellular adhesion molecule-1, MCP-1; monocyte chemoattractant protein-1, VCAM-1; vascular adhesion molecule-1, IL-8,6; interleukin-8 and 6, IL-1R antagonist; Interleukin 1 receptor antagonist, ROS; reactive oxygen species. Created with BioRender.com.

Additionally, Hcy upregulates MCP-1 (a protein that promotes monocyte adhesion to the endothelium and recruitment to the subendothelial cell compartment, which is an important stage in the progression of atherosclerotic lesions) expression via upregulating redox factor-1 (Ref-1) expression, activation of PKC and superoxide formation which activate NF-κB[44]. MCP-1's main mechanism of action is its contact with the MCP-1 receptor on the surface of monocytes (CCR2) which is stimulated by homocysteine, resulting in increased binding[46]. Also, homocysteine caused monocytes/macrophages to produce and secrete the tumor necrosis factor alpha (TNF-α), IL-1β, IL-8 and CCL2[41].

The migration of leukocytes from the vascular compartment into the tissue is a multistep process in Hcy-induced vascular inflammation. In the first step mediated by selectins, neutrophils are attracted to the vessel wall via margination, which is followed by rolling. Both neutrophils and endothelial cells express adhesion molecules and their ligands in response to certain stimuli, allowing for adherence and ultimate migration into tissues. Hcy stimulates the production of CD11B/CD18 proteins, which create a docking complex that allows inflammatory cells to interface with the endothelium. Additionally, Activation of mitogen-activated phosphokinases (MAPKs) in neutrophils and c-Jun NH(2)-terminal kinase in vascular endothelial cells has been indicated. Endothelial cells are damaged and lost as a result of the combined effects of these processes[42]. Moreover, homocysteine causes superoxide anion to be produced by NOX, which causes ERK1/2 and Akt signaling to be activated, resulting in the creation of neutrophil extracellular traps[47].

Also, through the activation of NF-κB, Hcy increases interleukin-6 (IL-6) expression in rat VSMC, and this increase in IL-6 exacerbates inflammation and consequent cellular impairment[48].

As Hcy increases the cholesterol synthesis; this stimulates LDL oxidation producing oxidized LDL (OxLDL). This increases endothelial LOX-1 (the main OxLDL receptor) gene expression, TNF-α release, MCP-1 and inhibits vasorelaxation[49]. OxLDL activates PPAR-γ in atherosclerotic lesion foam cells and modulates oxLDL another receptor CD36[50]. As a result, PPAR and CD36 activation creates a positive feedback loop that amplifies the effect of oxLDL. However, PPAR-α and PPAR-γ may decrease the expression of inflammatory gene in monocytes and result in anti-inflammatory response in the artery walls[51]. Moreover, OxLDL stimulates Nod-like receptor protein 3 (NLRP3) inflammasome activates inflammatory processes. Findings imply that NLRP3 inflammasome stimulation leads to HHcy-exacerbated inflammation and atherosclerosis in apoE -/- mice[52].

VEGF has also been linked to an increase in the size of atherosclerotic plaques[53]. Hcy increases VEGF expression, possibly by activating NF-κB. In human coronary atherosclerotic plaques, VEGF was reported to be generated in endothelial cells, smooth muscle cells and activated macrophages, and not in normal arteries[54]. Moreover, Increased expression of the receptor for advanced glycation end products (RAGE) leading to the production and accumulation of its signal-transducing ligand, EN-RAGE, further promotes the inflammation[48].

Evidence has emerged that ER stress triggers inflammatory responses via the PERK and IRE-1 pathways leading to generation of ROS and activation of NF-κB [55]. The acute-phase response in the liver is mediated by CREBH, a regulated intramembrane proteolysis (RIP)-regulated bZIP-containing transcriptional factor.[56] ER stress causes S1P and S2P proteases to cleave CREBH, releasing an amino-terminal fragment that travels to the nucleus and stimulates transcription of numerous gene including those encoding C-reactive proteins and serum amyloid P component which involved in the acute inflammatory process. It was shown that treatment with many ER stress inducers, such as thapsigargin, tunicamycin, A23187 and dextran sulphate, significantly boosted leukocyte adherence to smooth muscle cells. The hyaluronan structure was changed by ER stress to a more cable-like shape that was more favorable for leukocyte binding.[57]

CBS deficiency-induced HHcy has been found to promote inflammatory monocyte (ly6Chigh) differentiation, a mechanism that depends on superoxide anion generation. Moreover, Hcy- induced oxidative stress

increased the proliferation and activation of concanavalin A-stimulated splenic T-lymphocytes, indicating that T-lymphocytes were overactivated. It has been found that regulatory T-cells inhibit the inflammatory activation of T-lymphocytes and CTLA4-IgG reduced atherosclerotic lesions in apolipoprotein E-deficient (ApoE−/−) animals with HHcy via interfering with CD28 to prevent T-lymphocyte overactivation [41].

Homocysteine Promotes Coagulation

To prevent thrombosis, the endothelium maintains an equipoise between procoagulant substances and numerous antithrombotic processes. HHcy causes coagulation imbalance that leads to myocardial and peripheral thrombotic diseases **(Fig. 6,7),** which is predominantly caused by endothelial dysfunction[58]. According to reports, Hcy and HTL reduces the antithrombin III binding activity to cell surface in a dose and time dependent manner when aortic endothelial cells were incubated with different concentrations of Hcy[59].

Furthermore, Through Tissue Factor gene transcription (an integral membrane glycoprotein and forms a complex (1:1) with factor VII), Hcy initiates the coagulation TF pathway in diet-induced HHcy[29&59], and ECs that have been oxidatively disturbed increase the release of tissue factors like factor V, which transforms prothrombin to thrombin, and so commence pro-coagulant activity[60]. Moreover, GRP78 overexpression has been shown to reduce TF procoagulant activity, implying that inhibiting ER stress can suppress the prothrombotic activity[37].

As well, Hcy irreversibly suppresses the expression of thrombomodulin (a membrane-bound cofactor) on cell surface, which causes thrombin to be activated and reduces protein C activation[61].

At the same time, Hcy stimulates platelet adhesion by increasing the expression of Von Willebrand factor (VWF)[62]. Mild HHcy has been reported to enhance platelet sensitivity to adenosine diphosphate (ADP) and thrombin, resulting in platelet activation in individuals with peripheral vascular diseases **(Fig.7).** Aside from, ADP sensitivity boosted P-selectin expression. Phosphorylation of p38 mitogen-activated protein kinase (p38 MAPK),

cytosolic phospholipase A2 (cPLA2), as well as phospholipase C gamma 2 (PLC2) have all been implicated in the activation of platelets by HHcy[63]. Moreover, Luo et al, discovered that Hcy induces platelet activation by oxidized LDL and collagen type I via α2β1 and the glycoprotein VI pathway signaling components [64]. Hcy stimulates activation of platelets in humans and rats, and platelet aggregation by decreasing NO bioavailability and increasing thromboxane (TXA2) and prostaglandin H2 (PGH2) due to increased metabolism of arachidonic acid (AA), which induce platelet aggregation and vasoconstriction, both in platelets and the arteriolar endothelium [6365]. Hcy decreases NO which plays a major role in inhibition of plasminogen activator inhibitor-1 (PAI-1) expression and platelet adhesion as nitration of α-actin inhibits its phosphorylation[66]. HTL enhances platelet aggregation produced by thrombin in the same way that Hcy does, also, Hcy and HTL enhance platelet integrin αIIbβ3/ CD40L, which are implicated in platelet adhesion and aggregation[67,68].

Also, it was found that homocysteinylated LDL causes cytotoxicity in ECs. In contradiction,
homocysteinylated fibronectin reduces its interaction with fibrin, indicating a possible delay in hyperhomocysteinaemia and haemostasis[41].

Additionally, by a mechanism independent of TGF-α and TNF-β paracrine-autocrine activity, Hcy lowers cellular affinity for tissue plasminogen activator and increases gene expression and secretion PAI-1 from vascular endothelial and smooth muscle cells[69,59]. It has also been demonstrated that Hcy induce binding of lipoprotein A to fibrin, implying that thiol compound, thrombosis and atherosclerosis are all linked[70].

Because Hcy-SH is engaged in disulfide exchange or oxide-reduction reactions, it has been shown that Hcy improves plasma factor XIII-mediated fibrinogen crosslinking. Moreover, patients with mild HHcy generate clots that are more compact, have fewer and more often branching fibers, and are more resistant to fibrinolysis than those formed in the absence of Hcy, increasing the risk of atherothrombosis[67].
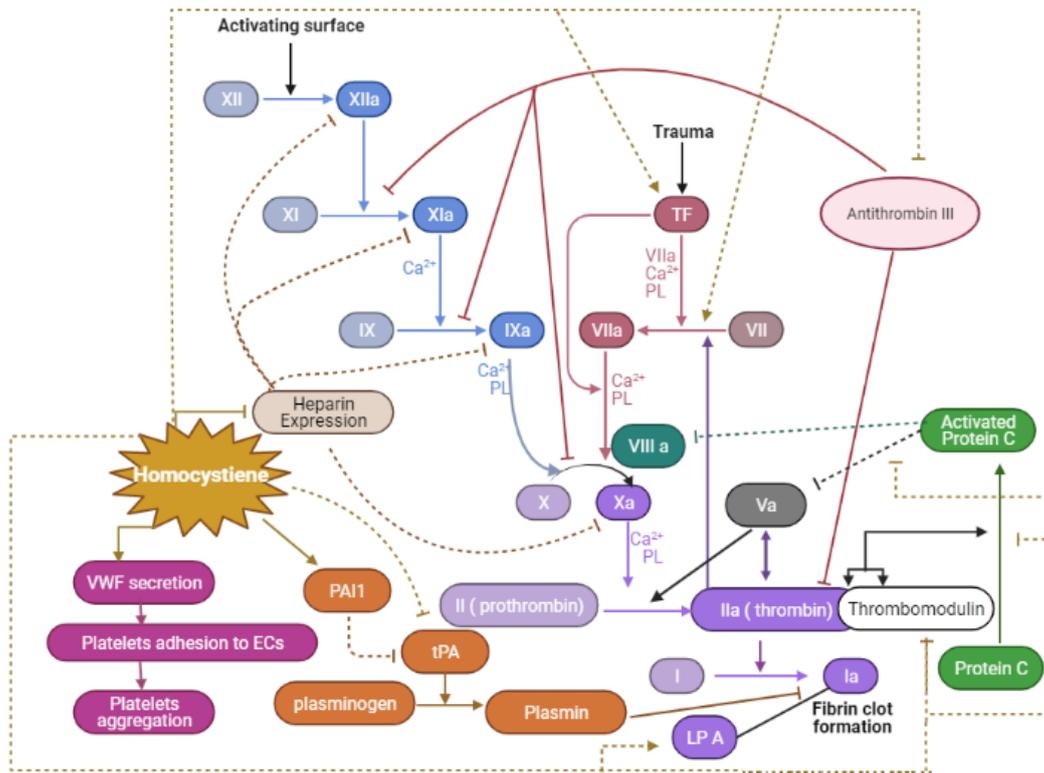
Fig. 6: *Homocysteine Promotes Coagulation. Created with BioRender.com*
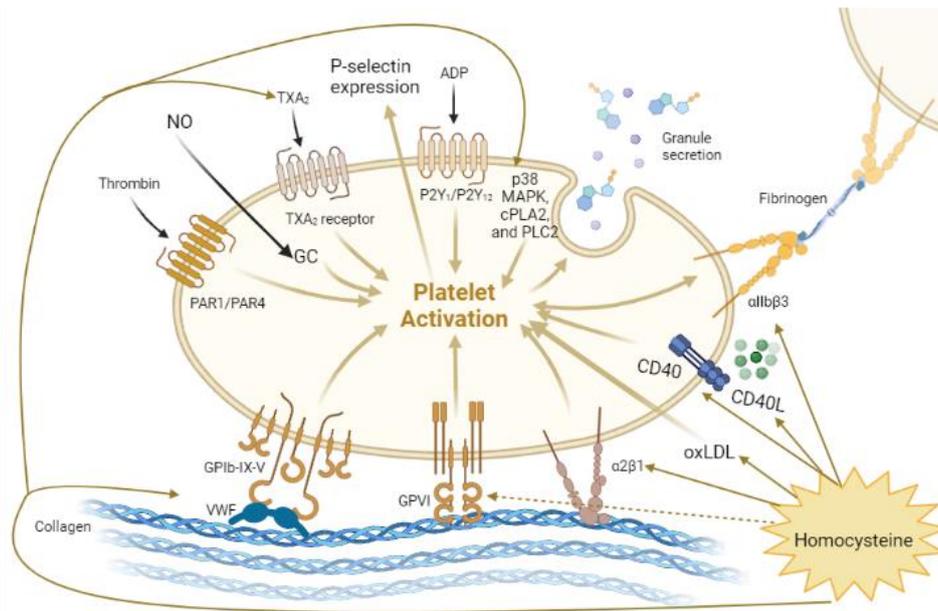
Fig.7: *Homocysteine promotes platelets activation. Created with BioRender.com*

Hcy or its thiolactone can covalently modify many proteins including fibrinogen. Homocysteinylation of twelve lysine residue was observed in fibrinogen treated with Hcy. several of them are near to the binding sites of plasminogen and tissue plasminogen activator (tPA), as well as plasmin cleavage sites. Moreover, a change in the C domain which could change the lateral connection of fibers and changes in protein conformation which may cause calcium binding to be

disrupted, resulting in changes in the structure of fibrin clot[67].

As a result, it's reasonable that high Hcy levels can cause thrombosis by boosting the procoagulant pathway and/or decreasing the anticoagulant pathway.

Endothelial fibrosis and SMCs proliferation

Vascular remodeling is described as an ongoing change in the size and composition of blood vessels and tissues that underpins the pathogenesis of major CVDs such as atherosclerosis. The degradation and synthesis of the Extra Cellular Matrix (ECM) are involved in both physiological and pathological vascular remodeling. Matrix metalloproteinases (MMPs), a group of specialized proteases, begin the process and regulate VSMCs proliferation[71].

Hcy-induced oxidative stress and reduced NO bioavailability stimulate expression and activity of MMPs impairing ECM metabolism and promoting collagen deposition, which result in fibrosis of blood vessels[72]. Hcy inhibits collagenase activity in the coronary arteries, resulting in collagen buildup and fibrosclerosis[73]. Fibrillar collagen plays an important role in the pathophysiology of atherosclerosis as it provides plaques with structural support. Arterial stenosis is caused by excessive collagen buildup whereas excess collagen degradation paired with insufficient production weakens plaques, making them more likely to rupture[74].

NO is known to be coordinated in MMPs' activation site, and to have a function in their latency. NO is quenched by Hcy-generated O2- leading to production of ONOO-, which uncovers the active site and increasing MMP activity[75].

Glomerulosclerosis mediated by high Hcy was observed in a diabetic mouse model with oxidative decrease of NO, and elevated MMP-2 activity, which was alleviated by activating PPARγ. It's thought that HHcy-dependent matrix buildup plays a role in hypertension-related vascular hypertrophy. Enhanced MMP-2 and MMP-9 activity contributes to ECM buildup and arterial remodeling in an animal model of HHcy-induced arterial hypertension[75].

Hcy promotes SMCs proliferation as Hcy increases AP-1 action by a Ca^{+2}-dependent pathway that boosts Erk action in SMCs,

influencing cells proliferation. Hcy has been shown to activate ATF4 to increase the production of VEGF mRNA P13-mediated activation of Akt/PKB is primarily responsible for the cell survival. Furthermore, VEGF causes the Raf-MEK-ERK pathway to be activated in human umbilical endothelial cells (HUVECs), which is linked to cell survival[59].

Apoptosis

By activating different death signaling pathways, HHcy promotes vascular damage by triggering apoptosis in a variety of cell types. The Fas cell death pathway, the p53/Noxa route, and the cytochrome-C activated caspases 3 and 9 pathway all have been demonstrated to be triggered by Hcy in ECs[37]. Furthermore, Hcy accelerated VSMC apoptosis mediated by caspase-3[76].

Hcy is also known to interfere with protein processing in the endoplasmic reticulum, resulting in the activation of unfolded proteins and ER stress, which leads to apoptosis[60]. Possibly by Hcy- induced peroxynitrite formation which has been shown to trigger apoptosis by a variety of mechanisms, including ER stress, a direct nitric oxide-dependent mechanism, p53/NADPH oxidase and activation of the MAPK/ERK and MAPK/JNK pathways[77].

Hcy- induced ER stress causes apoptosis dependent on IRE-1 signaling[78] and on the production of C/EBP homologous protein (CHOP is a growth arrest and DNA damage-inducible gene)/ GADD153[79]. Interaction between IRE-1 and TRAF2 (transduces signals from IREs that serve as stress sensors and begins UPR) stimulates apoptosis-signaling kinase (ASK1), which triggers apoptosis by stimulating JNK protein kinase leading to phosphorylation and activation of transcription factors including c-JUN. TRAF2 also connects to the TNFR-Associated Death Domain (TRADD), which attracts activated caspase 8, triggering a proteolytic cascade that leads to cell death. Caspases 8 also stimulates the secretion of the pro-apoptotic protein cytochrome C from the mitochondria[59].

Furthermore, the increased production of intracellular S-adenosylhomocysteine (SAH) caused by extracellular adenosine generated during Hcy biosynthesis promotes ECs death. SAH inhibits isoprenylcysteine

carboxylmethyl transferase (ICMT), which is responsible of Ras methylation leading to apoptosis. Likewise, L1210 cells undergo apoptosis when exposed to high levels of adenosine which is preceded by c-myc expression. C-myc expression makes cells vulnerable to a variety of pro-apoptotic stimuli, including hypoxia, DNA damage and starvation. The release of cytochrome C into the cytosol mediates the pro-apoptotic impact of c-myc . Apoptotic protease activating factor (APAF- 1) interacts with holocytochrome C, which recruits and activates procaspase 9. When cytochrome C is released into the cytosol, this attracts cells to other apoptotic triggers such as the CD95 pathway or p53 activation[59].

Moreover, by reducing the expression of cyclin A mRNA, Hcy may also impede ECs proliferation. In addition, cyclin dependent kinase (CDK2) activity was also suppressed considerably. Stress-induced induction of P53 stimulates expression of P21, which then interacts with CDKs and prevents the cell cycle G1/S transitioning.[59]

Homocysteine mediates lipids dysregulation

One of key mechanisms linking Hcy to atherosclerosis is Hcy-induced lipids disturbances. In hepatocytes, as well as vascular endothelial and aortic smooth muscle cells, Hcy-induced ER stress stimulates UPR, GRP78/BiP and sterol regulatory element-binding proteins (SREBPs)[80]. This process most likely indicates how liver steatosis and atherosclerosis lesions develop and worsen in HHcy[81].

Hcy stimulates SREBP-1 and SREBP-2 which regulate genes responsible for triglyceride biosynthesis and the expression of HMG-CoA reductase (3-hydroxy-3-methyl-glutaryl-coenzyme A reductase), the rate-limiting enzyme in cholesterol production. However, the actual transport of cholesterol in endothelial cells is downregulated, resulting in elevation of HMGCR in endothelial cells and increased cholesterol biosynthesis and accumulation [59]. Also, Hcy lower HDL cholesterol levels in plasma by blocking the hepatic synthesis of apoA-I, the primary HDL apolipoprotein[82].

Hcy was discovered to enhance macrophage lipoprotein lipase (LPL) expression (probably via PKC activation), as well as c-fos mRNA levels and nuclear protein binding to the AP1 sequence [83]. LPL is responsible for the breakdown of triglycerides in lipoproteins. In atherosclerotic lesions, macrophage LPL is released, and generated in the vascular wall works as a pro-atherogenic protein. This enzyme is involved in the absorption of lipoproteins by macrophages, the retention of lipoproteins in the ECM, the induction of the proatherogenic cytokine TNF-α, monocyte adherence to ECs, VSMCs proliferation as well as the production of foam cells[84].

Epigenetic modification in HHcy
First, Hypomethylation by Hcy limits ECs growth:

Hcy reduced cell growth in ECs by process known as hypomethylation [85]. HHcy-related MS or MTHFR insufficiency prevents Hcy from being converted into Methionine, thus reduces SAM formation. Whereas Hcy buildup raises the level of SAH and prevents SAM from being transmethylated. The reduced SAM/SAH ratio due to an increase in SAH and a decrease in SAM, coincides to the levels of global hypomethylation in cells[86]. In contrast, in CBS-deficient animals, Hcy caused decreased SAM/SAH ratio but with actually hypermethylation in liver and kidney. It was found that SAM's level did not drop but rather elevated, this is thought to be as a result of the functioning of MS and MTHFR[41].
Second, Hypomethylation by Hcy promotes VSMCs growth:

As a result of DNA demethylation mediated by the Hcy-reduced SAM/ SAH ratio, production of PDGF, a VSMCs proliferation inducer, is upregulated in VSMCs. Furthermore, SAM and SAH have opposed effects on VSMCs migration, proliferation, as well as neointimal formation. In obese diabetic rats, SAM protects from neointimal formation by inhibiting ER stress and VSMCs inflammation, while SAH boosts VSMCs migration and proliferation and increases atherosclerotic plaques through ERK1/2/pathway- dependent oxidative stress[41].

    b. Pathways of Hyperhomocysteinemia-induced cardiac dysfunction.

Hcy aggravates the cardiac remodeling, in addition to its atherosclerotic consequences. An unbalanced elevation of collagen in perivascular and interstitial, thickening of coronary arteriolar wall, and myocardial mast cell infiltration were seen in hyperhomocysteinemic rats' right and left cardiac ventricles[87]. The mechanisms of how Hcy- induced CHF are not fully understood, but it can be explained as follow:

Cardiac dysfunction caused by Hcy-induced vascular dysfunction:

Hcy is linked to coronary artery disease and myocardial infarction, the latter being a major cause of congestive heart failure. Hcy can also cause myocardial ischemia without an infarction by inducing endothelial dysfunction in coronary resistance arteries. In individuals with acute coronary syndrome, higher Hcy levels are linked to more myocardial damage, as demonstrated by higher troponin levels[88].

Hcy can cause acute negative inotropic effect and vasodilatory effect mediated by adenosine, which is released from the endothelium and blocked by endothelium inactivation[89]. Coronary dilation is mediated by an elevation of NO release or by the formation of S- nitroso- Hcy, a potent vasodilator, but other studies suggest that Hcy inactivates NO via oxidative mechanisms and impairs the synthesis of NO by the generation of ADMA[87]. It has been observed that nonselective Ado receptor antagonist inhibits the endothelium- dependent vasodilation induced by Hcy in the rat heart; they propose that Hcy releases Ado from endothelial cells, which then acts on the endothelium to release NO and relax VSM. However, this mechanism is likely distinct from the chronic effect of HHcy, which has been linked to elevated levels of both iNOS and eNOS, along with peroxynitrite formation[87].

Cardiac dysfunction caused by Hcy direct effect:

In hypertensive heart disease, cardiac remodeling is first characterized by remodeling of coronary arteriolar and myocardial matrix, which leads to higher myocardial rigidity and diastolic dysfunction. Then, systolic dysfunction and overt HF are seen after a time of compensated cardiac hypertrophy and diastolic dysfunction[87].

Myocardial collagen buildup was accompanied by an increase in the size of left ventricular myocytes in HHcy caused by heterozygous CBS deficiency. Parallel to fibrosis, a significant hypertrophy was seen in numerous cases[90]. It was observed that Hcy has a direct effect on myocardial fibrosis and systolic dysfunction, and the activated myocardial redox state could be a key factor of Hcy- induced cardiac remodeling. Also, Hcy is linked to cardiac mitochondrial dysfunction as Hcy promotes Ca^{+2} accumulation and oxidative stress in the mitochondria, boosting the activation of mitochondrial MMP, resulting in the opening of the mitochondrial permeability transition pore, leading to abnormal cardiac contractility, mechano-electrical dysfunction and perhaps arrhythmogenesis. Hcy also regulates endothelial and neuronal NOS, and so plays a role in cardiac arrhythmias[87].

Moreover, activated mitochondrial redox state results in liberation of cytochrome c from mitochondria. Caspases are activated by cytochrome c release, which results in the degradation of several nuclear proteins, which leads to DNA breakage and apoptosis[91].

Furthermore, mast cell is among the most prominent cellular aspects of Hcy-induced cardiac remodeling, according to certain studies, and it is associated with severe myocardial fibrosis[87].

As Hcy causes ER stress in the heart leading to myocardial inflammation, SREBP cleavage and apoptosis, as well as fibrosis[92]. SREBPs are transcription factors that are produced as inactive precursors and bound to ER membrane, and they are responsible for the upregulation of genes implicated in cholesterol production. SREBP-1a and -2 are the most common isoforms, but SREBP-1c and -2 are found in the majority of tissues. The overexpression of genes involved in cholesterol production is mediated by SREBP-1a and -2. SREBP-1c is required for the overexpression of genes involved in the fatty acid production pathway. SREBPs interact with SREBP cleavage activating protein (SCAP) and Insig to localize in the ER. SCAP interacts with SREBPs and is required for their activation. External free cholesterol is required for the survival of cells lacking SCAP. Insig is an ER-

resident protein anchor that binds SCAP and keeps the SCAP–SREBP complex in the ER[80&93].

Activation of caspases and apoptosis can occur as a result of prolonged or severe ER stress. Caspase-3 and -7 cleave SREBPs in response to apoptotic stimuli. SREBP's caspase cleavage site is thought to be on the N-terminal, cytoplasmic side. Caspase-3/7 cleaves SREBP at a different position than S1P/S2P, according to these findings. Other studies suggests that ER stress-induced SREBP activation is mediated by the site- 1 protease (S1P)/ site- 2 protease (S2P) proteolytic pathway (Colgan et al., 2007; Dorotea et al., 2020). Activation of SREBP as a result of ER stress leads to elevation of intracellular lipid levels and cardiac lipidopathy[80]. Elevated intracellular fat levels increase cardiac lipid lipolysis and fatty acid oxidation, leading to cardiac mitochondrial dysfunction and ER stress and forming a vicious loop of lipid biosynthesis–mitochondrial and ER stress leading to ventricular malfunction[92].

SREBP1 is considered as mediator of TGF-β1 signaling pathway[93]. TGFb1 may play a role in HHcy-induced heart structural remodeling. TGFb1 has been implicated in the development of fibrosis and ventricular hypertrophy in several studies. TGFb1 is known to promote a variety of signaling pathways, including the JNK, p38 and ERK pathways. In the hearts of HHcy rats, it was discovered JNK activation as well as elevated levels of MMP2 and TIMP2[90].

MMP-9 and MMP-2 activation by Hcy has previously been reported in ECs, VSM, and in the heart and aorta of CBS-deficient animals. Other findings of high MET- diet model demonstrate that rats with HHcy have no active MMP-9 form and have lower MMP-2 activity. This could be due to Hcy's cell type-specific effect or the etiology of HHcy (MET administration or enzyme deficiencies)[90]. MMP2 is released as a pro-enzyme and activated primarily through a membrane- linked process that involves MT1-MMP, while an excess of TIMP-2 restricts MMP-2 activation by attaching all MT1-MMP molecules. The increase in TIMP2 amount can then counteract the increase in MMP2 activity[90].

Conduction problems in infarction and heart failure appear to be linked to connexin dysfunction and prolonged action potential duration (APD), whereas in atrial fibrillation, APD reduction appears to be a prominent factor. Gap junction channels (GJCs), which are made up of connexins (Cxs), are responsible for intercellular communication. There are twenty-one human Cx isoforms, five of which are found in the heart: Cx31.9, Cx37, Cx40, Cx43, and Cx45. Cx43 is the most frequently expressed Cx in the heart, and it is found in both the atrial and ventricular working myocardium. Evidence suggests that long-term diet- induced HHcy suppresses Cx43 expression. In heart failure, Cx43 expression is downregulated, resulting in a positive feedback loop in which sluggish ventricular impulse conduction leads to mechanical dysfunction, increases ventricular remodeling and prolonged QRS complex[94-96].

Clinical Laboratory Evaluation of Hyperhomocysteinemia in Clinical Practice

The physician may suggest measuring Hcy plasma level if a patient displays symptoms of a vitamin B6, B12, or folate deficiency or if they are at a high risk of cardiovascular disorders or to diagnose homocystinuria which is a rare genetic disease. Temporary elevated blood level of Hcy can also be influenced by other factors, such as certain medications, heavy alcohol consumption, and smoking as previously mentioned. A Hcy test often does not require any particular preparation. The patient may be asked to fast for 8 to 12 hours. Additionally, the physician may advise the patient to stop using any supplements or medications before the test. Elevated Hcy plasma level may be a sign of: deficiency of vitamin B6, B12 or folic acid, homocystinuria, increased risk of heart diseases. Also, may occur in conjunction with other disorders, including osteoporosis, chronic renal disease, hypothyroidism, or Alzheimer's disease.

Total Hcy in plasma or serum is the sum of all homocysteine species, including free and protein-bound forms. Total Hcy can be detected in preserved samples using different method of assay whcich incorporate the reduction of all forms into a single species. Hcy is continuously produced and released from blood cells, which causes the total Hcy in whole blood to rise at room temperature. However, this increase is artificially reduced if

the blood sample is centrifuged within an hour of collection or stored on ice[97]. Many analytical approaches can be used to determine Hcy. Immunoassays including the enzyme immunoassay (EIA), chemiluminescence immunoassay (CLIA) and fluorescence polarisation immunoassay (FPIA). However, there has been a rise in interest in using chromatographic techniques[98].

Recent treatments of Hyperhomocysteinemia- induced cardiovascular dysfunction

Plasma concentration of Hcy may be lowered by giving cofactors of Hcy-metabolizing enzymes: folic acid, vitamin B6 and vitamin B12.

Reductions in oxidative stress may be responsible for the observed cardioprotective ability of Atorvastatin and Simvastatin, and statins may be used interchangeably to provide cardiac protection against I / R-induced myocardial injury in hearts of normal and hyperhomocysteinemic rats[99].

Recently, by stimulating the Ca2+ and Akt- eNOS- NO signalling pathways, Physcion inhibits Hcy-induced endothelial damage, showing that physcion could attenuate Hcy-induced cardiovascular disease[100].

Also, Homocysteine-responsive endoplasmic reticulum protein (Herp) deficiency inhibits the phenotypic transformation of VSMCs and the atherosclerosis [101].

Conclusion

High Hcy level can be a strong cause in several disorders including cardiovascular diseases. So, serious effort should be made to avoid and treat HHcy. All evidence suggests that Hcy is causally connected to atherosclerosis by oxidative stress, inflammation, endothelial-leukocyte interaction, coagulation, endothelial fibrosis, SMC proliferation, and apoptosis. But future studies should concentrate on the molecular pathways of HHcy in cardiac dysfunction, and the effects of Hcy-lowering medications on cardiac dysfunction.

REFERENCES

1. Z. Li, L. Lin, H.Wu, L. Yan, et *al.*, "Global, Regional, and National Death, and Disability-Adjusted Life-Years (DALYs) for Cardiovascular Disease in 2017 and Trends and Risk Analysis From 1990 to 2017 Using the Global Burden of Disease Study and Implications for Prevention", ***Front Public Health***, 9, 559751 (2021).

2. R. Ross, "Atherosclerosis — An Inflammatory Disease", ***New England Journal of Medicine***, 340(2), 115–126 (1999).

3. D. S. Wald, M. Law and J. K. Morris, "Homocysteine and cardiovascular disease: evidence on causality from a meta-analysis", ***BMJ***, 325(7374), 1202 (2002).

4. T. Rehman, M. A. Shabbir, M. Inam-Ur-Raheem, *et al.*, "Cysteine and homocysteine as biomarker of various diseases", ***Food Sci Nutr***, 8(9), 4696–4707 (2020).

5. G. Piazzolla, M. Candigliota, M. Fanelli, *et al.*, "Hyperhomocysteinemia is an independent risk factor of atherosclerosis in patients with metabolic syndrome", ***Diabetol Metab Syndr***, 11(1), 87 (2019).

6. A. Towfighi, D. Markovic and B. Ovbiagele, "Pronounced association of elevated serum homocysteine with stroke in subgroups of individuals: A nationwide study", ***J Neurol Sci***, 298(1–2), 153–157 (2010).

7. P. Chen, R. Poddar, E.V. Tipa, P.M .Dibello, *et al.*, "Homocysteine metabolism in cardiovascular cells and tissues: implications for hyperhomocysteinemia and cardiovascular disease", ***Adv Enzyme Regul***, 39(1), 93–109 (1999).

8. J. Selhub, "HOMOCYSTEINE METABOLISM", ***Annu Rev Nutr***, 19(1), 217–246 (1999).

9. P. R. Mandaviya, L. Stolk and S. G. Heil, "Homocysteine and DNA methylation: A review of animal and human literature", ***Mol Genet Metab***, 113(4), 243–252 (2014).

10. J. Kim, H. Kim, H. Roh and Y. Kwon, "Causes of hyperhomocysteinemia and its pathological significance", **Arch Pharm Res**, 41(4), 372–383 (2018).

11. P. Frosst, H.J. Blom, R. Milos, P. Goyette, C.A. Sheppard, *et al.*, "A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase", **Nat Genet**, 10(1), 111–113 (1995).

12. C. Tinelli, A. Di Pino, E. Ficulle, S. Marcelli and M. Feligioni, "Hyperhomocysteinemia as a Risk Factor and Potential Nutraceutical Target for Certain Pathologies", **Front Nutr**, 6,49 (2019).

13. R. Castro, I. Rivera, H. J. Blom, C. Jakobs and I. T. de Almeida, "Homocysteine metabolism, hyperhomocysteinaemia and vascular disease: An overview", **J Inherit Metab Dis**, 29(1), 3–20 (2006).

14. D. Leclerc, A. Wilson, R. Dumas, *et al.*, "Cloning and mapping of a cDNA for methionine synthase reductase, a flavoprotein defective in patients with homocystinuria", **Proc Natl Acad Sci U S A,** 95(6), 3059–3064 (1998).

15. D. Watkins, M. Ru, H.Y. Hwang, *et al.*, "Hyperhomocysteinemia Due to Methionine Synthase Deficiency, cblG: Structure of the MTR Gene, Genotype Diversity, and Recognition of a Common Mutation, P1173L", **Am J Hum Genet**, 71(1), 143–153 (2002).

16. A. Wilson, "Molecular basis for methionine synthase reductase deficiency in patients belonging to the cblE complementation group of disorders in folate/cobalamin metabolism", **Hum Mol Genet**, 8(11), 2009–2016 (1999).

17. B. A. Maron and J. Loscalzo, "The Treatment of Hyperhomocysteinemia", **Annu Rev Med**, 60(1), 39–54 (2009).

18. S. S. Qureshi, J. K. Gupta and N. Upmanyu, "A review on hyperhomocysteinemia and its risk factors", **Innovare J Agric Sci**, 4(1), 11–14 (2016).

19. R. Paul and A. Borah, "L-DOPA-induced hyperhomocysteinemia in Parkinson's disease: Elephant in the room", **Biochim Biophys Acta, Gen Subj**, 1860(9), 1989–1997 (2016).

20. Y. M. Smulders, A. M. de Man, C. DA Stehouwer and E. H. Slaats, "Trimethoprim and fasting plasma homocysteine", **The Lancet**, 352(9143), 1827–1828 (1998).

21. J. M. Ruscin, R. Lee Page and R. J. Valuck, "Vitamin B 12 Deficiency Associated with Histamine 2 -Receptor Antagonists and a Proton-Pump Inhibitor", **Annals of Pharmacotherapy**, 36(5), 812–816 (2002).

22. Q. Zhang, S. Li, L. Li,Q. Li, *et al.*, "Metformin Treatment and Homocysteine: A Systematic Review and Meta-Analysis of Randomized Controlled Trials", **Nutrients**, 8(12), 798 (2016).

23. J. B. Ubbink, A. v. der Merwe, R. Delport, R.H. Allen, *et al.*, "The effect of a subnormal vitamin B-6 status on homocysteine metabolism", **J Clin Investig**, 98(1), 177–184 (1996).

24. P. Durand, M. Prost, N. Loreau, S. Lussier-Cacan and D. Blache, "Impaired Homocysteine Metabolism and Atherothrombotic Disease", **Laboratory Investigation**, 81(5), 645–672 (2001).

25. M. Mydlík, K. Derzsiová and E. Žemberová, "Influence of Water and Sodium Diuresis and Furosemide on Urinary Excretion of Vitamin B6, Oxalic Acid and Vitamin C in Chronic Renal Failure", **Miner Electrolyte Metab**, 25(4–6), 352–356 (1999).

26. C. Desouza, M. Keebler, D. B. McNamara and V. Fonseca, "Drugs Affecting Homocysteine Metabolism", **Drugs**, 62(4), 605–616 (2002).

27. C. Desouza, M. Keebler, D. B. McNamara and V. Fonseca, "Drugs Affecting Homocysteine Metabolism", **Drugs**, 62(4), 605–616 (2002).

28. M. Barroso, D. E. Handy and R. Castro, "The Link Between Hyperhomocysteinemia and Hypomethylation", **J Inborn Errors Metab Screen**, 5, 232640981769899 (2017).

29. F. RH, W. BD, G. DB, F. LA and R. GM, "Homocysteine, a risk factor for premature vascular disease and thrombosis, induces

tissue factor activity in endothelial cells", ***Arterioscler Thromb***, 13(9), 1327–1333 (1993).

30. N. Weiss, Y.-Y. Zhang, S. Heydrick, C. Bierl, and J. Loscalzo, "Overexpression of cellular glutathione peroxidase rescues homocyst(e)ine-induced endothelial dysfunction", ***Proceedings of the National Academy of Sciences***, 98(22), 12503–12508 (2001).

31. F. G, B. T, N.-S. A, S. R, D. N, and C. G, "Structural modifications of HDL and functional consequences", ***Atherosclerosis***, 184(1), 1–7 (2006).

32. S. Dayal and S. R. Lentz, "ADMA and hyperhomocysteinemia", ***Vascular Medicine***, 10(2), S27–S33, (2005).

33. L. Jin, R. B. Caldwell, T. Li-Masters and R. W. Caldwell, "Homocysteine induces endothelial dysfunction via inhibition of arginine transport", ***J Physiol Pharmacol***, 58(2), 191–206 (2007).

34. Z. X, L. H, J. H, E. Z, B. S, and G. MS, "Effects of homocysteine on endothelial nitric oxide production", ***Am J Physiol Renal Physiol***, 279(4), F671-F678 (2000).

35. J. C. Barbato, O. Catanescu, K. Murray, P. M. DiBello and D. W. Jacobsen, "Targeting of Metallothionein by L-Homocysteine", ***Arterioscler Thromb Vasc Biol***, 27(1), 49–54 (2007).

36. J. Zhang, J. Liu, Z. Li, L. Wang, Y. Jiang and S. Wang, "Dysfunction of endothelial NO system originated from homocysteine-induced aberrant methylation pattern in promoter region of DDAH2 gene", ***Chin Med J (Engl)***, 120(23), 2132–2137 (2007).

37. N. Tyagi, K. C. Sedoris, M. Steed, A. V. Ovechkin, K. S. Moshal and S. C. Tyagi, "Mechanisms of homocysteine-induced oxidative stress", ***Am J Physiol Heart Circ Physiol***, 289(6), H2281-H2754 (2005).

38. P. A. Outinen, S. K. Sood, P. C. Y. liaw, *et al.*, "Characterization of the stress-inducing effects of homocysteine", ***Biochemical Journal***, 332(1), 213–221 (1998).

39. C. Ji, "Hyperhomocysteinemia, endoplasmic reticulum stress, and alcoholic liver injury", ***World J Gastroenterol***, 10(12), 1699 (2004).

40. I. J. Goldberg, "Lipoprotein lipase and lipolysis: central roles in lipoprotein metabolism and atherogenesis.", ***J Lipid Res***, 37(4), 693–707 (1996).

41. Y. Fu, X. Wang, and W. Kong, "Hyperhomocysteinaemia and vascular injury: advances in mechanisms and drug targets", ***Br J Pharmacol***, 175(8), 1173–1189 (2018).

42. S. Pushpakumar, S. Kundu and U. Sen, "Endothelial dysfunction: the link between homocysteine and hydrogen sulfide", ***Curr Med Chem***, 21(32), 3662–3672 (2014).

43. X. Zeng, J. Dai, D. G. Remick and X. Wang, "Homocysteine Mediated Expression and Secretion of Monocyte Chemoattractant Protein-1 and Interleukin-8 in Human Monocytes", ***Circ Res***, 93(4), 311–320 (2003).

44. J. Dai, W. Li, L. Chang, *et al.*, "Role of redox factor-1 in hyperhomocysteinemia-accelerated atherosclerosis", ***Free Radic Biol Med***, 41(10), 1566–1577 (2006).

45. R. F. Zanin, L. S. Bergamin, F. B. Morrone, R. Coutinho-Silva, A. T. de Souza Wyse, and A. M. O. Battastini, "Pathological concentrations of homocysteine increases IL-1β production in macrophages in a P2X7, NF-κB, and erk-dependent manner", ***Purinergic Signal***, 11(4), 463–470, (2015).

46. G. WANG and K. O, "Homocysteine stimulates the expression of monocyte chemoattractant protein-1 receptor (CCR2) in human monocytes: possible involvement of oxygen free radicals", ***Biochemical Journal***, 357(1), 233-240 (2001).

47. M. B. Joshi, G. Baipadithaya, A. Balakrishnan, *et al.*, "Elevated homocysteine levels in type 2 diabetes induce constitutive neutrophil extracellular traps", ***Sci Rep***, 6(1), 36362 (2016).

48. M. A. Hofmann, E.Lalla, Y. Lu, M R. Gleason, *et al.*, "Hyperhomocysteinemia enhances vascular inflammation and accelerates atherosclerosis in a murine model", ***J Clin Invest***, 107(6), 675–83 (2001).

49. K. B. Holven, H. Scholz, B. Halvorsen, P. Aukrust, L. Ose, and M. S. Nenseter, "Hyperhomocysteinemic Subjects Have Enhanced Expression of Lectin-Like Oxidized LDL Receptor-1 in Mononuclear Cells", *J Nutr*, 133(11), 3588–3591 (2003).

50. P. Tontonoz, L. Nagy, J. G. A. Alvarez, V. A. Thomazy and R. M. Evans, "PPARγ Promotes Monocyte/Macrophage Differentiation and Uptake of Oxidized LDL", *Cell*, 93(2), 241–252 (1998).

51. C. Jiang, A. T. Ting and B. Seed, "PPAR-γ agonists inhibit production of monocyte inflammatory cytokines", *Nature*, 391(6662), 82–86 (1998).

52. R. Wang, Y. Wang, N. Mu, X. Lou, *et al.*, "Activation of NLRP3 inflammasomes contributes to hyperhomocysteinemia-aggravated inflammation and atherosclerosis in apoE-deficient mice", *Laboratory Investigation*, 97(8), 922–934 (2017).

53. F. L. Celletti, J. M. Waugh, P. G. Amabile, A. Brendolan, P. R. Hilfiker, and M. D. Dake, "Vascular endothelial growth factor enhances atherosclerotic plaque progression", *Nat Med*, 7(4), 425–429 (2001).

54. M. Maeda, "Homocysteine induces vascular endothelial growth factor expression in differentiated THP-1 macrophages", *Biochimica et Biophysica Acta (BBA) - General Subjects*, 1623(1), 41–46 (2003).

55. K. Zhang and R. J. Kaufman, "From endoplasmic-reticulum stress to the inflammatory response", *Nature*, 454(7203), 455–462 (2008).

56. K. Zhang, X. Shen, Jun Wu, D. T. Rutkowski, *et al.*, "Endoplasmic Reticulum Stress Activates Cleavage of CREBH to Induce a Systemic Inflammatory Response", *Cell*, 124(3), 587–599 (2006).

57. A. K. Majors, R. C. Austin, C. A. de la Motte, *et al.*, "Endoplasmic Reticulum Stress Induces Hyaluronan Deposition and Leukocyte Adhesion", *Journal of Biological Chemistry*, 278(47), 47223–47231 (2003).

58. A. Coppola, G. Davi, V. De Stefano, F. P. Mancini, A. M. Cerbone and G. Di Minno, "Homocysteine, Coagulation, Platelet Function, and Thrombosis", *Semin Thromb Hemost*, 26(03), 243–254 (2000).

59. P. Sharma, R.D. Senthilkumar, V. Brahmachari, E. Sundaramoorthy,*et al.*, "Mining literature for a comprehensive pathway analysis: A case study for retrieval of homocysteine related genes for genetic and epigenetic studies", *Lipids Health Dis*, 5(1), 1 (2006).

60. D. W. Jacobsen, O. Catanescu, P. M. DiBello, and J. C. Barbato, "Molecular targeting by homocysteine: a mechanism for vascular pathogenesis", *Clinical Chemistry and Laboratory Medicine (CCLM)*, 43(10), (2005).

61. A. P. Selwyn, "Prothrombotic and antithrombotic pathways in acute coronary syndromes", *Am J Cardiol*, 91(12), 3–11 (2003).

62. E. MM and C. JA, "Hyperhomocysteinemia and thrombosis: an overview", *Arch Pathol Lab Med*, 131(6), 872–884 (2007).

63. T. Huang, G. Yuan, Z. Zhang, Z. Zou and D. Li, "Cardiovascular pathogenesis in hyperhomocysteinemia", *Asia Pac J Clin Nutr*, 17(1), 8–16 (2008).

64. F. Luo, X. Liu, S. Wang and H. Chen, "Effect of homocysteine on platelet activation induced by collagen", *Nutrition*, 22(1), 69–75 (2006).

65. M. M. Steed and S. C. Tyagi, "Mechanisms of Cardiovascular Remodeling in Hyperhomocysteinemia", *Antioxid Redox Signal*, 15(7), 1927–1943 (2011).

66. S. Marcondes, M. H. M. Cardoso, R.P. Morganti, *et al.*, "Cyclic GMP-independent mechanisms contribute to the inhibition of platelet adhesion by nitric oxide donor: A role for -actinin nitration", *Proceedings of the National Academy of Sciences*, 103(9), 3434–3439 (2006).

67. K. Karolczak and B. Olas, "Mechanism of action of homocysteine and its thiolactone in hemostasis system", *Physiol Res*, 623–633 (2009).

68. C. Prontera, N. Martelli, V. Evangelista, E. D'Urbano, *et al.*, "Homocysteine

Modulates the CD40/CD40L System", *J Am Coll Cardiol*, 49(22), 2182–2190 (2007).

69. K. A. Hajjar, "Homocysteine-induced modulation of tissue plasminogen activator binding to its endothelial cell membrane receptor", *J Clinical Invest*, 91(6), 2873–2879 (1993).

70. P. C. Harpel, V. T. Chang and W. Borth, "Homocysteine and other sulfhydryl compounds enhance the binding of lipoprotein (a) to fibrin: a potential biochemical link between thrombosis, atherogenesis, and sulfhydryl compound metabolism", *Proceedings of the National Academy of Sciences*, 89(21), 10193–10197 (1992).

71. F. H. Epstein, G. H. Gibbons, and V. J. Dzau, "The Emerging Concept of Vascular Remodeling", *New England J Med*, 330(20), 1431–1438 (1994).

72. U. Sen, P. K. Mishra, N. Tyagi and S. C. Tyagi, "Homocysteine to hydrogen sulfide or hypertension", *Cell Biochem Biophys*, 57(2–3), 49–58 (2010).

73. J. W. Heinecke, L. Baker, H. Rosen and A. Chait, "Superoxide-mediated modification of low density lipoprotein by arterial smooth muscle cells", *J Clinical Invest*, 77(3), 757–761 (1986).

74. P. Libby, "Molecular Bases of the Acute Coronary Syndromes", *Circulation*, 91(11), 2844–2850 (1995).

75. A. Radomski, G. Sawicki, D. M. Olson, M. W. Radomski, "The role of nitric oxide and metalloproteinases in the pathogenesis of hyperoxia-induced lung injury in newborn rats", *Br J Pharmacol*, 125(7), 1455–1462 (1998).

76. S. S. Mok, B. J. Turner, K. Beyreuther, C. L. Masters, C. J. Barrow, , and D. H. Small, "Toxicity of substrate-bound amyloid peptides on vascular smooth muscle cells is enhanced by homocysteine", *Eur J Biochem*, 269(12), 3014–3022 (2002).

77. F. Dong, X. Zhang, S.h.i-Yan Li, *et al.*, "Possible Involvement of NADPH Oxidase and JNK in Homocysteine-Induced Oxidative Stress and Apoptosis in Human Umbilical Vein Endothelial Cells", *Cardiovasc Toxicol*, 5(1), 009–020, (2005).

78. C. Zhang, Y.Cai, M. T. Adachi, *et al.*, "Homocysteine induces programmed cell death in human vascular endothelial cells through activation of the unfolded protein response", *J Biol Chem*, 276(38), 35867–35874 (2001).

79. P. A. Outinen *et al.*P. A. Outinen *et al.*, "Characterization of the stress-inducing effects of homocysteine.", *Biochem J*, 332 (Pt 1, 213–21, (May 1998).

80. S. M. Colgan, D. Tang, G. H. Werstuck and R. C. Austin, "Endoplasmic reticulum stress causes the activation of sterol regulatory element binding protein-2", *Int J Biochem Cell Biol*, 39(10), 1843–1851 (2007).

81. G. H. Werstuck, S. R. Lentz, S. Dayal, G. S. Hossain, S. K. Sood, *et al.*, "Homocysteine-induced endoplasmic reticulum stress causes dysregulation of the cholesterol and triglyceride biosynthetic pathways", *J Clinical Invest*, 107(10), 1263–1273 (2001).

82. P. J. Barter and K.-A. Rye, "Homocysteine and Cardiovascular Disease", *Circ Res*, 99(6), 565–566 (2006).

83. M.-C. Beauchamp and G. Renier, "Homocysteine Induces Protein Kinase C Activation and Stimulates c-Fos and Lipoprotein Lipase Expression in Macrophages", *Diabetes*, 51(4), 1180–1187 (2002).

84. Y. Pirahanchi, M. Anoruo, and S. Sharma, *"Biochemistry, Lipoprotein Lipase"*, (2021). [Online]. Available: http://www.ncbi.nlm.nih.gov/pubmed/30725725

85. M. E. Lee and H. Wang, "Homocysteine and hypomethylation. A novel link to vascular disease", *Trends Cardiovasc Med*, 9(1–2), 49–54 (1999).

86. P. K. Kamat, C. J. Mallonee, A. K. George, S. C. Tyagi and N. Tyagi, "Homocysteine, Alcoholism, and Its Potential Epigenetic Mechanism", *Alcohol Clin Exp Res*, 40(12), 2474–2481 (2016).

87. E. Vizzardi, I. Bonadei, G. Zanini, *et al.*, "Homocysteine and heart failure: an overview*", Recent Pat Cardiovasc Drug Discov*, 4(1), 15–21 (2009).

88. R. S. Vasan, "Plasma Homocysteine and Risk for Congestive Heart Failure in Adults Without Prior Myocardial Infarction", ***JAMA***, 289(10), 1251 (2003).

89. R. H. Kennedy, R. Owings, N. Shekhawat and J. Joseph, "Acute negative inotropic effects of homocysteine are mediated via the endothelium", ***Am J Physiol Heart Circ Physiol***, 287(2), H812–H817 (2004).

90. L. Raaf, C. Noll, M. El Hadi Cherifi, *et al.*, "Myocardial fibrosis and TGFB expression in hyperhomocysteinemic rats", ***Mol Cell Biochem***, 347(1–2), 63–70, (2011).

91. L. J, "The oxidant stress of hyperhomocyst(e)inemia", ***J Clin Invest***, 98(1), 5–7, (1996).

92. J. P. Ayyappan, K. Lizardo, S. Wang, E. Yurkow and J. F. Nagajyothi, "Inhibition of SREBP Improves Cardiac Lipidopathy, Improves Endoplasmic Reticulum Stress, and Modulates Chronic Chagas Cardiomyopathy", ***J Am Heart Assoc***, 9(3), e014255, (2020).

93. D. Dorotea, D. Koya, and H. Ha, "Recent Insights Into SREBP as a Direct Mediator of Kidney Fibrosis via Lipid-Independent Pathways", ***Front Pharmacol***, 11, 265 (2020).

94. C. Soni, "Hyperhomocysteinemia alters sinoatrial and atrioventricular nodal function : role of the cardiac NMDA receptor", ***University of Louisville,*** (2012).

95. E. G. J. Vermeulen, M. I. A. van Engeland, F. C. Visser, C. D. A. Stehouwer, *et al.*, "Effect of homocysteine-lowering vitamin treatment on electrocardiography stress tests in a randomized, placebo-controlled trial: comparison between ST-segment changes and Athen QRS-score", ***Int J Cardiol***, 93(2–3), 323–324 (2004).

96. A. Miller, V. Mujumdar, L. Palmer, J. D. Bower, and S. C. TyagiA, "Reversal of endocardial endothelial dysfunction by folic acid in homocysteinemic hypertensive rats", ***Am J Hypertens***, 15(2), 157–163 (2002).

97. P. M. Ueland, H. Refsum, S. P. Stabler, M. R. Malinow, A. Andersson, and R. H. Allen, "Total homocysteine in plasma or serum: methods and clinical applications", ***Clin Chem***, 39(9), 1764–1779 (1993).

98. Ł. Paprotny, D. Wianowska, M. Izdebska, A. Celejewska, D. Szewczak, and J. SolskiŁ, "Analysis of serum homocysteine in the laboratory practice - comparison of the direct chemiluminescence immunoassay and high performance liquid chromatography coupled with fluorescent detection.", ***Biochem Med (Zagreb)***, 30(3), 030703 (2020).

99. A. Rohilla, A. Ahmad, M. U. Khan, and R. Khanam, "A comparative study on the cardioprotective potential of atorvastatin and simvastatin in hyperhomocysteinemic rat hearts", ***Eur J Pharmacol***, 764, 48–54 (2015).

100. X.W. Ji, H. J. Lyu , G. H. Zhou , B. Wu, *et al.*, "Physcion, a tetra-substituted 9,10-anthraquinone, prevents homocysteine-induced endothelial dysfunction by activating Ca2+- and Akt-eNOS-NO signaling pathways", ***Phytomedicine***, 81, 153410 (2021).

101. H. Lin, T. Ni, J. Zhang, L. Meng, *et al.*, "Knockdown of Herp alleviates hyperhomocysteinemiaf mediated atherosclerosis through the inhibition of vascular smooth muscle cell phenotype switching", ***Int J Cardiol***, 269, 242–249 (2018).

نشرة العلوم الصيدلية

جامعة أسيوط

فرط الهوموسستئين في الدم: تأثيره على أمراض القلب والأوعية الدموية وتصلب الشرايين

إسراء جلال* – عبير الخولي – شيماء محمد – أماني أحمد

قسم علم الأدوية والسموم، كلية الصيدلة ، جامعة حلوان، القاهرة ، مصر

أمراض القلب والأوعية الدموية هي السبب الرئيسي للوفاة في كل من البلدان النامية والمتقدمة. فرط الهوموسستيين في الدم هو عامل خطر لتصلب الشرايين وأمراض القلب والأوعية الدموية .تصلب الشرايين هو حالة التهابية مزمنة يتراكم فيها الكوليسترول والرواسب الدهنية والكالسيوم فـي طبقــة البطانة ، مما يضيق الشرايين فرط الهوموسستيين هي حالة طبية تتميز بتركيــز غيــر طبيعــي مــن الهوموسيستين ، وهو حمض أميني غير بروتيني يحتوي على الكبريت ويوجد في بلازما الــدم بشـكل طبيعي، وقد عثر على أنه يتسبب في العديد من الأمراض.

يمكن أن ينتج فرط الهوموسيستيين نتيجة لأحد الأسباب التالية:

١. الاختلافات الجينية في الإنزيمات المهمة مثل MS و MTRR و MTHFR وCBS

٢. نقص التغذية للعديد من العوامل المساعدة في التمثيل الغذائي للهوموسيستيين

٣. الإفراط في استخدام بعض الأدوية

٤. العرقية والجنس والعمر وبعض الأمراض

تهدف الدراسة الحالية إلى: جمع وتلخيص ما تم دراسته من مسارات فرط الهوموسيستيين والتي تؤدي إلى حدوث تصلب الشرايين ومشاكل القلب المزمنة. كما تهدف الدراسة الحالية إلـى الوقــوف علــى العلاجات التي تمت دراستها للتقليل من فرط الهوموسيستيين وماينتج عنه مــن مشـاكل فـي القلـب والأوعية الدموية.

ولتحقيق هذه الأهداف: تمت مراجعة كل الأبحاث الصادرة في هذا المجال وتلخيص ما ذكر فيها.

يمكن تلخيص نتائج هذا البحث كما يلي: يمكن أن يسبب فرط الهوموسيستيين تصلب الشرايين وخلل في وظائف القلب من خلال الإجهاد التأكسدي والالتهاب والتفاعل البطاني مع الكريات البـيض والتخثـر والتليف البطاني وتكاثر الخلايا العضلية وموت الخلايا المبرمج واختلال تنظـيم الـدهون والتعـديلات اللاجينية وتعديلات التوصيل الكهربائي في القلب. ثبت أن بعض الأدوية التي تمت دراستها فعالة فـي تقليل مستوى الهوموسيستيين. لكن يجب أن تركز الدراسات المستقبلية على المسارات الجزيئة لـفرط الهوموسيستيين في اختلال وظائف القلب ، وتأثيرات الأدوية المخفضة للهوموسيستيين علـى ضـعف القلب.