Hyperhomocysteinemia (Hhcy) is a biochemical abnormality characterized by abnormally large levels of homocysteine (Hcy) in blood. Hcy is a highly reactive sulfur-containing amino acid derived from methionine, an essential amino acid. Excess Hcy produced in the body is excreted out of the tightly regulated cell environment into the blood. It is the role of the liver and kidney to remove excess Hcy from the blood. Hcy increases the generation of reactive oxygen species by activating nicotinamide adenine dinucleotide phosphate oxidase. It downregulates the endothelial nitric oxide (NO) synthase and thus reduces the bioavailability of NO. Moreover, it increases the production of proinflammatory cytokines like tumor necrosis factor-α by activating nuclear factor-kappa B. Hcy is caused by the excess deficiencies of the vitamins such as pyridoxine (B6), folic acid (B9), or B12 and is considered as an independent risk factor for various cardiovascular diseases such as endothelial dysfunction, vascular inflammation, atherosclerosis, hypertension, cardiac hypertrophy, and heart failure. Many evidences are available which suggest that ignoring Hcy levels in patients with the vascular disease would be unwise. Thus, there is an urgent need for health care providers to develop effective preventions and interventions program (follic acid, Vitamin B6, and Vitamin B12 supplementation as well as lifestyle change) to reduce this disorder.

**Keywords:** Homocysteine, Stroke, Folic acid, Vitamin B6, Vitamin B12, Atherosclerosis.

**INTRODUCTION**

Hyperhomocysteinemia (Hhcy) is a disorder characterized by an abnormal increased level of homocysteine (Hcy) in the blood, above 15 μmol/L [1]. Jukes reported that Hcy is a sulfur-containing amino acid, isolated from a urinary bladder stone in 1933 by Vincent du Vigneaud [2]. It has been under a lot of speculation since its discovery. Its chemical property showed a similarity to cysteine; hence, the name is Hcy. Hcy is an amino acid produced via demethylation of dietary methionine, which is abundant in animal protein [3,4]. It is the role of the liver and kidney to remove excess Hcy from the blood. People with Hhcy get blood clots in their veins and arteries (e.g., deep vein thrombosis and pulmonary embolism). Hcy is a key determinant of the methylation cycle. It is metabolized either by remethylation pathway to methionine or the transsulfuration pathway to cysteine. Condensation of methionine with adenosine triphosphate (ATP), leads to the formation of s-adenosylmethionine (SAM), a principle methyl donor for all methylation reactions in cells [5,6]. The demethylation reaction leads to the formation of s-adenosylhomocysteine (SAH) which further leads to the formation of Hcy and adenosine. The former pathway of Hcy metabolism is dependent on the proper functioning of methylene tetrahydrolfolate reductase (MTHFR) enzyme, methionine synthetase, Vitamin B12, and folic acid. The later pathway is dependent on the enzymes cystathionine beta-synthetase and MTHFR [7].

The prevalence of Hhcy varies widely with geography, sex, ethnicity, and age, for example, Amouzou and coworkers reported that coastal West Africa people had a higher prevalence of Hhcy than the people located in an interior part of countries. The incidence of Hhcy in the American population is just 5-7%, in Chinese is 27.5%, and in Indians is 52-84%. Indians were found to have higher Hcy levels than Europeans. It is estimated that mild Hhcys occur in 5-7% of the general population and 40% of patients with vascular disease. The incidence of homocystinuria in the United States is approximately 1 per 100,000. In 1990-98, stroke mortality in the US was falling at 0.3% a year but after folate substitution in 1998, the mortality dropped to 2.9% per year, a 10 times change. The frequency of polymorphism (common mutation in MTHFR gene) is very low in some populations (<1% in African descent) and very high in others (11-15% in Anglo-Americans, >20% in Italian, Hispanic, and Columbians and 25-57% in Mexican population). Bangladeshi men have a high prevalence of Hhcy, which is associated with smoking and betel nut use [8,9].

**CAUSES OF HHCY**

**Dietary factor**

Hcy elevation occurs because of poor diet (absence of essential food components). If a person takes high protein rich diet daily, i.e., meat, egg, chicken, milk, etc., he should also incorporate certain vital amines in his diet. The absence of these vital amines causes Hhcy [10].

**Life style factors**

Smoking is associated with vascular disease and other complications related to Hhcy. The number of cigarettes smoked a day is the strongest determinant of Hcy levels. Nicotine directly affects the methylation and catabolises folate cycle. Physical activity plays an important role in life since it is the cheapest way of strengthening our health and reduces the risk of cardiovascular diseases (CVD). Physical inactivity decreases the concentrations of total plasma Hcy, and thus increases the probability of developing CVD in healthy and already sick people. Hcy increases with age, changes in gastrointestinal function, B Vitamins deficiency, enzyme defects, and a higher occurrence of the C677T mutation occur in elderly [11].

**Vitamin deficiency**

Vitamin B6, B12, and folic acid are needed for the enzymes involved in Hcy metabolism; high protein intake seems to lower Hcy levels. Strict vegetarians are often at risk for Hhcy due to low plasma B12 levels. Coffee consumption (4 cups/day) is linked to moderate elevations in Hcy, although this effect can be countered by supplementing with 200 mg/day of folic acid. Diagnosis is made by measuring the level of total Hcy in the blood. Patients having the diseases such as dyslipidemia, diabetes, renal insufficiency, Alzheimer disease, and hypertension should be tested for Hhcy. Hcy is tested by chromatographic method, immunoassay method, enzyme cycling method, capillary electrophoresis, and chemosensors. Blood vessels abnormalities, atherosclerosis, mental retardation in children, thrombosis, and bone loss are common symptoms of Hhcys [12,13].
**Alcohol induced**
Chronic intake of alcohol consumption interferes in the metabolism of folic acid and cyanocobalamin. It is associated with gastrointestinal disturbances, which result in decreased absorption of vitamins and folic acid, thus contributing to elevated Hcy levels. It also inhibits methionine synthase to decrease hepatic uptake and increase excretion via urine.

**Drug induced**
Certain drugs such as cholestyramine and metformin prevent vitamin absorption from the gut. Methotrexate, nicotinic acid, and fibrin acid derivatives interfere with folate and Hcy metabolism. Oscarbazepine and topiramate might cause Hcy because of their capacity to activate hepatic enzymes.

**Renal dysfunction**
Renal failure patients have extremely high Hcy levels due to less efficient renal clearance of Hcy. Patients with kidney disease have high rates of cardiovascular morbidity and death is observed. It exhibits disproportionately elevated plasma with Hcy levels. Hcy levels increase as renal function declines. The underlying cause of Hcy in renal disease is not yet understood, although reduced plasma Hcy clearance is the most proximate cause [14,15].

**Genetic factors**
Hcy is caused either by genetic defects in the enzymes which are involved in Hcy metabolism or by nutritional deficiencies in vitamin cofactor. Genetic defects in genes encoding for enzymes such as methyl tetrahydrofolate (THF), methionine synthase, and cystathionine-β-synthase causes a deficiency. Methyl THF enzyme is responsible for the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. German and Italian population show different incidences of 24.5% and 43.8% of genetic defects in genes encoding for these enzymes. Certain rare defects in this gene cause homocystinuria, brain damage, and childhood CVD [16].

**MOBILIZATION OF HCY PATHWAY**
Hcy is a sulfur-containing amino acid which is generated from the metabolism of methionine, the metabolism of which involves four steps. The first step is transmethylation pathway which involves the conversion of methionine to Hcy [17]. This pathway involves the formation of SAM, which transfers a methyl group to a number of several methyl acceptor molecules (proteins, DNA, neurotransmitters) and forms adenosylhomocysteine, which is subsequently converted to Hcy. The second step is the transsulfuration pathway that involves the irreversible conversion of Hcy to cysteine in the presence of cystathionine-β-synthase and Vitamin B6 as an essential cofactor. The third step is the re-methylation pathway in which regeneration of methionine occurs from Hcy by methionine synthase along with MTHF – 5 Methyltetrahydrofolate and Vitamin B12 as essential cofactors. The last step is the regeneration of methyltetrahydrofolate (MTHF) from THF, which is catalyzed by enzyme 5, 10-methlene-tetrahydrofolate reductase [18,19] (Fig. 1).

**TREATMENT**
The internationally accepted treatment for Hcy involves the use of vitamins, i.e. folic acid, Vitamin B12, and pyridoxine. Folic acid and vitamin predominantly act under fasting condition and pyridoxine acts after meals. Pyridoxine reduces Hcy levels by 22%. Folic acid alone reduces Hcy level by 22% and Vitamin B12 by 11%. When both administered together, it causes a reduction of 38.5%. Pyridoxine (10,250 mg/d) lowers an abnormal Hcy level in most patients [20]. Daily dose of 100 mg or less can precipitate peripheral neuropathy [21]. Treatment also includes anticoagulant medications such as aspirin, clopidogrel, heparin, warfarin, to prevent blood clots. Amount of methionine diet is restricted. Atorvastatin (10 mg) along with three Hcy lowering vitamins (methylcobalamin 500 mcg, folic acid 5 mg, and pyridoxine 10 mg) improved in diabetic patients. Daily intake of (400-800 μg/day folic acid, 500 μg/day B12, and 25-100 mg/day B6).

**RISK FACTORS**

**Cardiac impairment**
The relationship between Hcy and atherosclerosis was suggested by McCully in 1969. It is now well accepted that Hcy is a strong, independent risk factor for stroke, myocardial infarction, and other vascular events [24]. Hcy is an unstable amino acid, which undergoes auto oxidation to produce free oxygen radicals which further increases oxidative stress. It contributes to atherosclerosis in two ways. The former one includes free oxygen radicals converts low-density lipoproteins of sub endothelial tissues to oxidized low-density lipoproteins (LDL). Oxidized LDL further acts as a key mediator of the inflammatory process in atherosclerosis. Oxidized LDL causes the release of vascular cell adhesion molecule and monocyte chemotactic protein [25-27]. The monocytes get converted to macrophages, which take up oxidized LDL to get converted to foam cells. The foam cells get deposited below the endothelium to form fatty streak. The latter part includes the suppression of nitric oxide activation by free radicals, which results in endothelial dysfunction and contributed to atherosclerosis [28].

**Stroke**
Stroke is a major cause of morbidity and mortality worldwide. Along with hypertension, dyslipidemia, smoking, diabetes mellitus (DM), obesity, and family history, Hcy is one such risk factor for stroke. There is epidemiological evidence suggesting a relationship between Hcy and neurodegenerative conditions including Alzheimer, Parkinson disease, and stroke [29]. Energy metabolism and oxidative stress are related to the pathogenesis of this disease. Energy demands of central nervous system are fulfilled with continuous supplies of oxygen and glucose from the blood flow. Increased Hcy level reduces ATP availability for neurons and causes severe neuronal injury. The disrupted metabolism of Hcy is associated mainly with tissue hypoxia and vascular disease [30]. Liver, kidney, and lens cells have the ability to convert Hcy to methionine through a B12-independent reaction catalyzed by betaine homocysteine methyltransferase (BHMT). This reaction requires dietary betaine or endogenous choline. Central nervous system lacks BHMT, being thus dependent on folate and
Vitamin B12 for the conversion of Hcy to methionine. Hcy is an important endothelial aggression factor which promotes endothelial dysfunction and leads to atherosclerotic plaque formation. It inhibits the growth of endothelial cells, induces an imbalance between oxygen and NO with deleterious effects on the vascular physiology. It induces the expression of different adhesion molecules and promotes the formation of modified LDL particles, which are important players in atherosclerotic plaque formation [31].

Cancer risk
For the past several years, a link has been established between certain cancers and elevated plasma Hcy. Increased plasma Hcy concentration is a risk factor for cancer and even as a novel tumor marker [32]. Folate depletion promotes the development of cancer, particularly colorectal cancer 6-10, whereas high doses of folic acid enhance the growth of cancer cells. Folate, Vitamin B12, and Vitamin B6 have a number of biologic roles that make them potentially important in cancer. Hcy is the most common condition highly associated with both venous and arterial thrombosis in many cancer patients while the associated pathophysiology has not been established yet. Defective metabolism of Hcy in carcinogenesis is well documented, but the pathophysiology is not fully understood [33,34]. North American and Western European societies have a high risk of developing cancer due to low intake of vegetables and particularly folic acid in diets. Folic acid deficiency enables DNA damage to occur and accumulate. This can lead to DNA damage and cancer [35].

Diabetes mellitus
Hhcy is common in many diabetic patients and may contribute to the accelerated risk of atherosclerosis and CVD. The adverse vascular effect of Hcy in diabetes appears to be related primarily to type 2 diabetes mellitus (T2DM). In the west, the incidence of Hhcy in diabetic patients is reported to be approximately 5 times greater than the general population [27,36,37]. Hhcy is detected in patients with DM, which may contribute to the development of chronic complications. It correlates with both changes in glomerular filtration rate as well as the presence of microalbuminuria. Hcy concentrations correlate with the presence of diabetic peripheral neuropathy and have also been associated with the presence of autonomic neuropathy in patients with type 1 diabetes. Hcy elevation in patients with DM only occurs when renal function deteriorates. The influence of diabetes treatment on Hcy levels requires further advance observations. Therapy with insulin and medications such as metformin and gliptones can either raise or lower Hcy levels. Hhcy is known to be a risk factor for vascular occlusive diseases. Metsä et al. reported that Hhcy is an independent risk factor of CVD incidence in diabetic patients. Hhcy increased the risk of CVD by 1.6 times in diabetic patients. It increases the mortality in T2DM [38,39].

CONCLUSION
Over the past decade, Hcy related research provoke a huge amount of scientific literature and sparked a vigorous debate, as an emerging risk factor for neural tube defects (NTD) and non-communicable disease (NCDs), including type 2 diabetes and cancer. Kimner MC Cully, in 1960, concluded that mutation in Hcy related gene leads to increased risk of CVD. Certain drugs (adenosine analogs, d-penicillamine, N-acetylcysteine, estrogens, tamoxifen, and betaine) decrease blood Hcy concentration through different mechanisms (i.e., remethylation stimulation, inhibition of SAH hydrolase activity). In past two decades, numerous studies have been conducted in developed countries to explore Hcy status and its determinants. The result of these studies suggests that it could be caused by environmental exposures, lifestyles habits, disease status, hormonal factors, and several drugs [40]. Patients with heart failure and documented Hhcy should be considered for therapy with folic acid, vitamins and increased intake of fruits and vegetables since treatment is effective, inexpensive, without risk, and may lower the risk of adverse cardiac events. Patients with heart failure impaired renal function, and diabetes should be screened since the incidence of Hcy in these patients appears to be quite high.