

# Hyperhomocysteinemia as a risk factor for coronary heart diseases in chronic hepatitis C patients

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## ABSTRACT

Hepatitis C virus is one of the major health problems worldwide. It affects mainly the liver but several extrahepatic manifestations are also accounted. Chronic hepatitis C patients are at an increased risk of developing hepatic steatosis, which share many clinical features with the metabolic syndrome. Hepatic steatosis has also been associated with elevated levels of markers of inflammation such as homocysteine, identified as hyperhomocysteinemia (HHC). HHC due to Methyltetrahydrofolate Reductase (MTHFR) gene, in particular the C677T polymorphism, was recently associated with coronary heart diseases (CHD) in chronic hepatitis C (CHC) patients. Homocysteine is an intermediate in methionine metabolism, which takes place mainly in the liver metabolism. Deficiencies of micronutrients (folate, vitamin B 6 and possibly vitamin B 12) along with mild hyperhomocysteinemia, perhaps, act synergistically with other classical risk factors to further increase the risk of CHD. Clinical data indicate that HHC is associated with an increased incidence of CHD as well as with the severity of the disease in CHC patients. In conclusion, HHC might be a potential aetiological factor of CHD in CHC patients. The aim of this review is to investigate the progression of coronary heart diseases in chronic hepatitis C patients and correlate with levels of homocysteine in concurrence to genetic defects and nutrient deficiencies. However, future studies need to clarify the mechanistic role of HHC in CHD and CHC as a useful paradigm with most interesting therapeutic implications.

**Keywords:** Hepatitis C Virus; Hyperhomocysteinemia (HHC); Coronary Heart Diseases (CHD); Chronic Hepatitis C (CHC)

## 1. INTRODUCTION

Chronic infection with hepatitis C virus (HCV) is one of the leading causes of chronic liver disease; about 170 million people worldwide are estimated to be infected. Hepatitis C virus infection causes acute symptoms in only 15% of patients exposed to HCV infection while about 80% patients develop chronic infection [1]. Chronic hepatitis C results in formation of high levels of free radicals in the liver cells, which put serious oxidative stress depleting protective antioxidants and eventually kill the liver cells. A hepatitis screen is recommended for patients whereby the disease can be diagnosed by the presence of antibodies for hepatitis C or by the direct presence of the virus or viral products in the blood [2].

## 2. ASSOCIATED RISKS: CHRONIC HEPATITIS C

Hepatitis C virus (HCV) infection is a major cause of chronic liver disease. HCV infection frequently does not resolve, leading to chronic hepatitis with increasing risk of developing hepatic fibrosis, steatosis, liver cirrhosis, hepatocellular carcinoma, metabolic syndromes, arthrosclerosis and extrahepatic diseases [3]. The combination of pegylated interferon (IFN)- $\alpha$  and ribavirin is the only treatment for chronic HCV infections with proven efficacy.

Unfortunately, this therapeutic strategy results in a low sustained virologic response (SVR), defined as an absence of detectable serum HCV-RNA at six months after completion of antiviral therapy; SVR is achieved in less than 50% of treated patients that have HCV genotype 1 and a high viral load [4]. There is evidence indicating that SVR is associated with long-term clearance of HCV infection and lower HCV-related complications [5,6]. However, IFN- $\alpha$  in combination with ribavirin is generally not well tolerated, and the adverse side effects may

lead to interruption or cessation of therapy. The major adverse effects are anemia, fatigue, hair loss, depression, insomnia, vertigo, anorexia, nausea, nasal congestion, cough, dyspnea, pruritus, and growth delay [7].

### 3. HEPATITIS C VIRUS AND CARDIAC RISK

Chronic hepatitis C virus (CH-C) infection is associated with metabolic conditions such as insulin resistance and type 2 diabetes (T2DM) and may increase the risk of coronary heart diseases. Coronary artery disease is the most common heart disease with multifactorial etiology. Atherosclerosis being the principal cause has plagued human kind since ancient times. Its understanding has much evolved over centuries, traditionally being viewed as degenerative disease, is now considered a dynamic inflammatory and fibroproliferative process, triggered by cytokines and growth factors [8-12]. In addition to other conventional atherogenic risk factors (Age, Sex, Smoking, Hypertension, Diabetes Mellitus and Dyslipidaemia), one of the most interesting development in the recent years has been the idea that infective agents may induce a pro-inflammatory state and have a crucial role in atherothrombosis [12-14].

### 4. INADEQUATE VITAMINS AND HCV

Patients with chronic HCV infection have significantly lowered plasma vitamin B1, B2, B6, C, and folic acid levels [15]. These patients were also observed to have significantly higher plasma homocysteine (a sulfur-containing amino acid, which is influenced by vitamin B2, 6, 12, and folic acid) concentrations and lower concentrations of folic acid and vitamin B12 [16]. The plasma homocysteine levels were inversely correlated with the concentrations of folic acid in HCV-infected patients. SVR patients have been observed to have lower plasma homocysteine levels than non-SVR patients [17]. Pre-treatment with IFN- $\alpha$  and ribavirin in chronic HCV-infected patients, serum vitamin B12 levels are positively correlated to end-of-treatment response [18].

Several infectious etiologies for coronary heart diseases (CHD) have been proposed in recent years on the basis of epidemiological associations, but there is no consensus regarding a causative role [19-21]. The association between hepatitis C virus (HCV) infection and CHD is less clear. A small number of reported studies have shown conflicting results; some have reported no association between HCV infection and CHD [22-25], whereas others have reported an increased risk [26] or an increase in measures of subclinical atherosclerosis (e.g., carotid intima-media thickness) [26-28]. Many of the studies showing no association between HCV infection and CHD used a case-control design in which subjects

with known CHD were compared with control subjects without CHD and the prevalence of HCV infection was compared between the 2 groups without adjustment for all Coronary artery diseases (CAD) risk factors. However, Persons with HCV infection are at an increased risk of developing hepatic steatosis, which shares many clinical features with the metabolic syndrome [29,30]. Hepatic steatosis has also been associated with elevated levels of markers of inflammation and endothelial dysfunction [31]. Hepatitis C virus increases the risk of coronary artery disease, a large American study published in the Clinical Infectious Diseases [32]. These factors suggest a biologically plausible mechanism of increased risk of CHD in at least a subset of HCV-infected persons.

The main rationale of this review is shown in **Figure 1**.

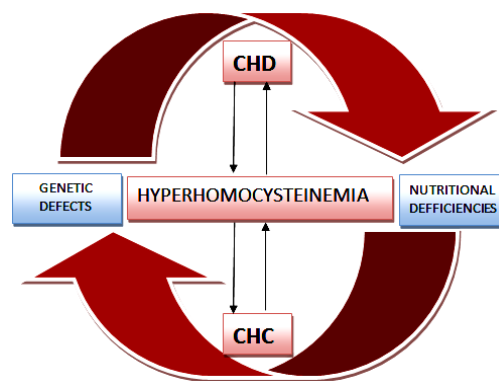
### 5. HOMOCYSTEINE METABOLISM

Homocysteine (Hcy) belongs to a group of molecules known as cellular thiols. It is considered a “bad thiol” because its association with a variety of health conditions including cardiovascular disease, [33] end-stage renal disease, [34] neural tube defects, [35]. Recently, homocysteine has also been implicated in the pathogenesis of alcoholic liver injury [36].

The 5,10-methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in the folate cycle and contributes to the metabolism of the amino-acid homocysteine. It irreversibly catalyzes the reduction of 5,10-methylene-tetrahydrofolate to 5-methyltetrahydrofolate, the major circulatory form of folate in the body and a carbon donor for conversion of homocysteine to methionine, precursor of S-adenosyl-L-methionine [37].

### 6. HOMOCYSTEINE AND CHRONIC HEPATITIS C

Homocysteine levels are also altered in chronic liver disease. Homocysteine is a sulphur containing amino acid belonging to the group of intracellular thiols. Numerous



**Figure 1.** Rationale of study.

clinical and epidemiological studies have reported that elevated plasma homocysteine concentrations reflect impaired cellular metabolism [38] and may be considered as an independent risk factor for atherosclerotic vascular disease and thromboembolism [39]. Experimental data in transgenic mice deficient in homocysteine metabolism enzymes have shown the presence of severe liver steatosis with occasional steatohepatitis. In human beings, many studies have found a correlation between homocysteine and steatosis [40].

Homocysteine is mainly synthesized and metabolized in the liver, since metabolism of majority of dietary methionine occurs in this organ, where about 85% of the whole body capacity for transmethylation resides. Therefore, genes involved in methionine and homocysteine metabolism are expressed in a specific pattern in the liver [41]. Homocysteine is formed as an intermediate in methionine metabolism; therefore, impaired liver function leads to altered methionine and homocysteine metabolism [42]. Plasma homocysteine levels were significantly elevated in HCV infected patients in both sexes compared with control values. These findings are in accordance with the results of many studies observed elevated plasma homocysteine levels in patients with liver cirrhosis secondary to hepatitis C virus infection [38]. Some of the studies have attributed this condition to reduced expression of genes involved in Hcy metabolism. The degree of reduced expression of these genes was related to the severity of liver disease [40].

Alterations in Hcy metabolism in human liver cirrhosis can be ascribed in part to a marked reduction in the expression of the main genes involved in its metabolism, namely methionine synthase (MS) and betaine-homocysteine methyltransferase (BHMT), which convert homocysteine back to methionine, and cystathionine-synthase (CBS), the first enzyme in the transsulfuration pathway [41]. The expression of these genes was always more compromised than that of HSA and was related to the severity of the disease, expressed as the Child-Pugh score. We observe reduced expression of Hcymetabolizing genes, both in alcoholism and hepatitis C virus cirrhosis. It has been suggested that impairment of Hcy metabolism in cirrhosis most possibly can be also related to decreased availability or utilization of vitamins B6, B12, or folates, [43].

## 7. HYPERHOMOCYSTEINEMIA

Elevated levels of homocysteine, Hyperhomocysteinemia (HHC), may result from defects in homocysteine-metabolizing genes; (such as MTHFR, Methylene tetrahydrofolate Reductase gene) vitamin B6, B12, or folate deficiencies resulting from nutritional conditions; or chronic alcohol consumption [40]. The hyperhomocysteinemia is known as atherogenic and thrombotic risk factor

for cardiovascular disease. It might also be a risk factor for cirrhotic patients but the direct effect of Hcy on liver injury is not well known [44].

Hyperhomocysteinemia was correlated with elevated levels of ALT, ALP, TGs and cholesterol. This might be related to progression of liver injury. Some other studies have also reported a correlated elevation of plasma Hcy levels with ALT, ALP, TGs and cholesterol [45,46]. It is evident that homocysteine-induced endoplasmic reticulum stress leaves a dysregulated endogenous sterol response pathway, which leads to increased hepatic biosynthesis and uptake of cholesterol and triglycerides [47].

## 8. GENETIC DEFECTS IN HOMOCYSTEIN METABOLISM

Elevations in plasma homocyst(e)ine are typically caused either by genetic defects in the enzymes involved in homocysteine metabolism or by nutritional deficiencies in vitamin cofactors. Homocystinuria and severe hyperhomocyst(e)inemia are caused by rare inborn errors of metabolism resulting in marked elevations of plasma and urine homocyst(e)ine concentrations. Cystathionine *b*-synthase deficiency is the most common genetic cause of severe hyperhomocyst(e)inemia. The homozygous form of this disease—congenital homocystinuria—can be associated with plasma homocyst(e)ine concentrations of up to 400 mmol per liter during fasting [48]. The homozygous trait is rare (occurring in 1 in 200,000 births), and clinical manifestations include ectopia lentis, skeletal deformities, mental retardation, thromboembolism, and severe, premature atherosclerosis [49]. Atherothrombotic complications frequently develop in young adulthood in homozygotes and are often fatal, as first shown in a study by Carey and colleagues as early as 1968 [49]. Mudd and colleagues [50] have estimated that approximately 50 percent of untreated patients with homocystinuria will have a thromboembolic event before the age of 30 and that overall, the disease-related mortality is approximately 20 percent.

Heterozygotes typically have much less marked hyperhomocyst(e)inemia, with plasma homocyst(e)ine concentrations in the range of 20 to 40 mmol per liter, approximately two to four times greater than the normal concentration of homocyst(e)ine in plasma [49,51-53]. A homozygous deficiency of N5, N10-methylenetetrahydrofolate reductase, the enzyme involved in the vitamin B12-dependent remethylation of homocysteine to methionine, may also lead to severe hyperhomocyst(e)inemia [54]. Patients with this type of deficiency tend to have a worse prognosis than those with cystathionine *b*-synthase deficiency, in part because of the complete lack of effective therapy [55,56]. In addition, studies (Kang and colleagues) [57] have reported a thermolabile

variant of N5, N10-methylene-tetrahydrofolate reductase that is caused by a point mutation (MTHFR\_C677T) in the coding region for the N 5, N 10-methylene-tetrahydrofolate binding site, leading to the substitution of valine for alanine [58].

## 9. MTHFR: C→T POLYMORPHISM

A single nucleotide polymorphism (SNP) in the MTHFR gene, which is located in the chromosome 1p36.3, has been identified. A C-to-T transition at the nucleotide 677 (C677T) in exon four results in an alanine to valine exchange which affects the catalytic domain of the enzyme; as a consequence, a thermo-labile variant of MTHFR, called t-MTHFR, is synthesized, which possesses reduced enzyme activity [59].

One of the most common mutations, or polymorphisms, that are associated with a mild increase in plasma homocysteine (hyperhomocysteinemia) is the 677C→T substitution (an alanine to valine change) in the enzyme methylenetetrahydrofolate reductase (MTHFR). The MTHFR is an enzyme of the folate metabolism that reduces 5,10-methylenetetrahydrofolate (5,10-mTHFR) to 5-methyltetrahydrofolate (5-mTHF), an important co-factor to homocysteine (Hcy) methylation. Mutations in MTHFR gene like C677T result in amino acids substitutions that lead to a decreased enzyme activity [60,61]. As a consequence of the MTHFR dysfunctions, an increased Hcy level in plasma has been expected which, in turn, produces a cytotoxic effect [62].

## 10. CONCLUSION

Hepatitis C virus infection is a major cause of progressive liver damage whose long term sequelae includes cirrhosis and primary hepatocellular carcinoma. HCV mainly affects the liver, but several tissues outside the liver have been reported to be involved, resulting in a wide spectrum of extrahepatic manifestations. Despite having fewer risk factors for cardiovascular disease, the hepatitis C-infected individuals were more likely to have been diagnosed with coronary artery disease. Multiple prospective and case-control studies have shown that a moderately elevated plasma homocysteine concentration is an independent risk factor for atherothrombotic vascular disease. Homocysteine concentrations are consistently higher in patients with peripheral, cerebrovascular, and coronary artery disease than in those without such diseases. Homocyst(e)ine promotes atherothrombogenesis by a variety of mechanisms; however, it is not yet clear whether homocysteine itself or a related metabolite or cofactor is primarily responsible for the atherothrombotic effects of hyperhomocyst(e)inemia *in vivo*. However, it is biologically plausible that hepatitis C may increase the risk of disease such as heart attack and stroke

as hepatitis steatosis (fatty liver), a common complication of hepatitis C infection, has been associated with increased levels of homocysteine and metabolic syndrome. Though, the reason(s) and mechanism(s) of this association need further study.”

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