# Asymmetric Dimethyl Arginine in Chronic Active Hepatitis

**Thesis**

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# Abstract

Asymmetric dimethyle arginine(ADMA) has an important role in regulating NO production by acting as competitive inhibitor of all three NOS isoforms.

ADMA is synthesized during the methylation of protein arginine residues by protein arginine methyltransferases (protein methylases, PRMT).

The aim of this study was to evaluate plasma ADMA level in patients with chronic active hepatitis and to correlate its level with liver dysfunction.

Fifty five subjects were included in this study and were classified into 15 healthy subjects as control (group I), 20 chronic active hepatitis C patients with no sonographic evidence of cirrhosis( group II) and 20 chronic active hepatitis C patients with sonographic evidence of cirrhosis (group III).

Plasma ADMA level of was measured by ELISA.

This study showed that

Hepatic dysfunction is the most prominent determinant of ADMA plasma concentration.Highly significant increase in the mean plasma ADMA level in cirrhotic group as compared to both control group and non cirrhotic group. Significat correlation between plasma ADMA and serum ALT, total bilirubin and albumin.



**Key words**:

Assymetric dimethyl arginine, Nitric oxide, Chronic active hepatitis.

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| Abbrev | Full name |
|---|---|
| ADC | Arginine decarboxylase |
| ADMA | Asymmetric dimethyl arginine |
| AGAT | Arginine glycine amidinotransferase |
| ARG | Arginase |
| ASL | Argininosuccinate lyase |
| ASS | Argininosuyccinate synthetase |
| AST | Aspartate transaminase |
| $BH_4$ | Tetrahydrobiopterin |
| CAH | Chronic active hepatitis |
| CAM | Calmodulin |
| Enos | Constitutive nitricx oxide synthase |
| DAG | Diacylglycerol |
| DDAH | Dimethylarginine dimethy aminohydrolase |
| eNOS | Endothelial nitric oxide synthase |
| ER | Endoplasmic reticulum |
| FAD | Flavin adenine dinucleotide |
| FMN | Flavin mononucleotide |
| GDP | Guaosine diphosphate |
| GTP | Guanosine triphosphate |
| HBV | Hepatitis B virus |
| HCV | Hepatitis C virus |

| | |
|---|---|
| HDV | Hepatitis D virus |
| iNOS | Inducible nitric oxide synthase |
| $IP_3$ | Inositol triphosphate |
| IR | Ionotropic receptor |
| NADPH | Nicotinamide adenine dinucleotide phosphate |
| NO | Nitric oxide |
| $NO^-_2$ | Nitrite |
| $NO^-_3$ | Nitrate |
| NOS | Nitric oxide synthase |
| OAT | Ornithine aminotransferase |
| OTC | Ornithine transcarbamylase |
| $P_5C$ | L-$A^1$- pyrroline –5-carboxylate |
| PLCβ | Phospholipase Cβ |
| PRMTS | Protein arginine methyl transferases |
| SDMA | Symmetric dimethyl arginine |

# Introduction

The arginine-nitric oxide pathway has been recognized to play a crucial role during infection, inflammation, organ injury and transplant rejection. Nitric oxide (**NO**) is synthesized from arginine by the action of nitric oxide synthases(**NOS**), a family of enzymes with endothelial, neuronal and inducible isoforms **(Furchgott, 1996)**.

Experimental data indicate that NO suppresses platelet aggregation (**Wolf et al, 1997**),leukocyte adherence **(Tsao et al, 1997**) and vascular smooth muscle proliferation **(Von der leyen et al, 1995**).

In the immune system, NO is a regulator of immune cell function. NO is shortyly lived, being degraded into nitrite and nitrate. The synthesis of nitric oxide is inhibited by endogenous arginine analogues: asymmetric dimethyl arginine (**ADMA**),symmetric dimethyl arginine (**SDMA**) and N-monomethyl arginine, the most abundant being ADMA (**Vallence et al,1992**).

Methyl arginines, are synthesized from arginine by the action of enzymes known as protein arginine methyl transferases (**PRMTS**). Proteolysis of proteins containing the methylated arginine residues releases free methyl arginine products into the cytosol from where they pass to plasma (**Leiper and Vallence,1999)**.

ADMA acts as an inhibitor of NOS (**Leiper and Vallence,1999)**, while SDMA has no inhibitory effect on NOS, but may interfere with NO synthesis by competing with L-argnine for transport across cell membranes (**Goonasekra et al,1997)**.

ADMA is excreted in part by the kidneys but the main metablic pathway is degradation by two dimethylarginine dimethylaminohydrolases ( **DDAH-I and II** ) to L citrulline and dimethylamine (**Leiper et al,1999**). However, SDMA is eliminated by renal clearance and cannot be degraded by DDAH. The enzyme DDAH is highly

expressed in the live (**Ogawa et al, 1998)**.

It was shown, in rats, that the liver takes up larg amounts of ADMA from the systemic circulation **(Nijvelt et al, 2003a**). Thus the liver may play a major role in determining the plasma levels of ADMA.Theoretically, dysfunction of the liver may disturb dimethylarginine metabolism. Indeed, in critically ill patients, hepatic dysfunction has proven to be the most prominent determinant of ADMA plasma concentration (**Nijveldt et al , 2003b**).

## Aim of the study

The study is planned to evaluate plasma level of ADMA in patient with chronic active hepatitis and correlate its level with liver dysfunction.

# 1-Chronic Hepatitis

The term chronic hepatitis means active, ongoing inflammation of the liver persisting for more than six months that is detectable by biochemical and histological means **(Ishak, 1994)**.

The biochemical hallmark of chronic hepatitis is an increased serum aspartate aminotransferase and alanine aminotransferase (AST and ALT) with minimal elevation of alkaline phosphatase. When the inflammation is severe and/or prolonged, hepatic dysfunction may become apparent with an increase in serum bilirubin and prothrombin time, and a decrease in serum albumin.Typically, biochemical tests are used to identify and follow patients with chronic hepatitis, while liver biopsies serve to more precisely define the nature of the chronic hepatitis and provide useful information regarding the extent of damage and prognosis **(Desmet et al, 1994)**.

Histologically, chronic hepatitis is characterized by infiltration of the portal tracts by inflammatory cells.These cells are predominantly mononuclear cells including lymphocytes, monocytes and plasma cells.

Chronic hepatitis is designated as mild when the infiltrate is confined to the portal triad. It is designated as moderately severe chronic hepatitis when the infiltrate extends into the parenchyma (piecemeal necrosis) and when it extends to adjacent portal triads (bridging).The inflammatory process can also "bridge" from the portal tract to the central vein. Severe chronic hepatitis is associated with multilobular or confluent necrosis and is much more likely to progress to cirrhosis **(Ishak et al, 1995)**.

These terms, mild, moderate and severe chronic hepatitis, replace the older terminology including chronic persistent hepatitis and chronic active hepatitis, which

are still frequently mentioned in older textbooks **(Czaja, 1993 and Batts and Ludwig, 1995)**.

By far, the commonest cause of chronic hepatitis is viral infections of the liver.Other causes include autoimmune hepatitis, drug-induced hepatitis, Wilson's disease, $\alpha_1$-antitrypsin deficiency and steatohepatitis **(Scheuer, 1995)**.

**Clinical manifestations:**

Clinical features vary widely. About $^1/_3$ of cases develop after acute hepatitis. Many patients are asymptomatic, especially in chronic hepatitis C viral infection (HCV).However, malaise, anorexia, and fatigue are common, sometimes with low-grade fever and nonspecific upper abdominal discomfort. Often, particularly with HCV, the first findings are signs of chronic liver disease, splenomegaly, spider nevi, palmar erythema **(Gerber, 1992)**. In the autoimmune variant, especially in young women, manifestations may involve virtually any body system and can include acne, amenorrhea, arthralgia, ulcerative colitis, pulmonary fibrosis, thyroiditis, nephritis, and hemolytic anemia **(Johnson, McFarlane, 1993).**

Chronic HCV is occasionally associated with mucocutaneous vasculitis, glomerulonephritis, porphyria cutanea tarda, and, perhaps, non-Hodgkin B-cell lymphoma.

**Diagnosis**

The diagnosis is suspected in patients with suggestive symptoms and signs, incidentally noted elevations in aminotransferase levels, or previously diagnosed acute hepatitis. Liver function tests are needed if not previously done and include serum ALT, AST, alkaline phosphatase, and bilirubin. Aminotransferase elevations are the most characteristic laboratory abnormalities. **(Desmet et al, 1994)**.

Although levels vary, in chronic hepatitis may reach 100 to 500 IU/L, ALT is usually higher than AST. Aminotransferase levels can be normal during chronic hepatitis if the disease is quiescent, particularly with HCV**(McFarlane,1993)**. Alkaline phosphatase is usually normal or only slightly elevated but is occasionally markedly high. Bilirubin is usually normal unless the disease is severe or advanced. However, abnormalities in these laboratory tests are not specific and can result from other disorders, such as alcoholic liver disease, acute viral hepatitis, and primary biliary cirrhosis.

If laboratory results are compatible with hepatitis, viral serologic tests are done to assess viral etiology. Further testing may be required for other etiology.The first tests done include autoantibodies, immunoglobulins, and $\alpha_1$-antitrypsin level. Children and young adults are screened for Wilson's disease with a ceruloplasmin level. Marked elevations in serum immunoglobulins suggest chronic autoimmune hepatitis but are not conclusive. Autoimmune hepatitis is normally diagnosed based on the presence of antinuclear (ANA), anti-smooth muscle, or anti-liver/kidney microsomal type 1 (anti-LKM1) antibodies at titers of 1:80 (in adults) or 1:20 (in children) **(Zachou et al, 2004).**

Mild cases may have only minor hepatocellular necrosis and inflammatory cell infiltration, usually in portal regions, with normal acinar architecture and little or no fibrosis. Such cases rarely develop into clinically important liver disease or cirrhosis.

In more severe cases, biopsy typically shows periportal necrosis with mononuclear cell infiltrates (piecemeal necrosis) accompanied by variable periportal fibrosis and bile duct proliferation. The acinar architecture may be distorted by zones of collapse and fibrosis, and frank cirrhosis sometimes coexists with signs of ongoing hepatitis. Biopsy is also used to grade and stage the disease **(Colombari et al, 1993)**.

In most cases, the specific cause of chronic hepatitis cannot be discerned via