Serum Hepcidin Levels In Patients With Chronic Hepatitis C

Thesis

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By

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Abstract

Background

Patients with chronic hepatitis C (CHC) often have increased liver iron, a condition associated with reduced sustained response to antiviral therapy, more rapid progression to cirrhosis, and development of hepatocellular carcinoma. The hepatic hormone hepcidin is the major regulator of iron metabolism and inhibits iron absorption and recycling from erythrophagocytosis. Hepcidin decrease is a possible pathophysiological mechanism of iron overload in CHC, but studies in humans have been hampered so far by the lack of reliable quantitative assays for the 25-amino acid bioactive peptide in serum (s-hepcidin).

Aims

To determine the clinical relevance of hepatic producing iron regulatory hormone-hepcidin, on iron overload in patients with chronic hepatitis C (CHC). And To detect the effect of interferon based therapy on hepcidin level correlating it with early virological response (This may be later used as a predictor of response to interferon therapy according to hepcidin levels).

Patient&Methods

Serum hepcidin measured in 30 CHC patients by surface-enhanced laser desorption/ionization time of flight mass spectrometry (SELDI-TOF-MS), and compared to those of healthy controls and analyzed their relationship to the clinical, hematological, and histological findings. The sequential changes of hepcidin were investigated in CHC patients treated with a 24 weeks-course of pegylated-interferon (PEG-IFN) plus ribavirin therapy.

Results

Serum hepcidin was significantly lower in CHC patients compared to the control group Serum hepcidin was positively correlated with serum ferritin. Serum hepcidin-to-ferritin ratios were significantly lower in HCV positive patients than in HCV negative controls . This relative impairment of hepcidin production was fully reversible after 24 weeks courseby PEG-IFN plus ribavirin.

Conclusions

The consequences of dysregulation of the hepcidin expressionby HCV may be an important mechanism underlying the iron overload seen in CHC and may have significant implications for the management of chronic HCV infection. Improvement for its regulation or supplementation of hepcidin may be beneficial for CHCpatients with iron overload.

Keywords: Chronic hepatitis C, Iron, Iron-regulated genes, Interferon, Ribavirin, Surface-enhanced laser desorption/ionization time of flight mass spectrometry (SELDI-TOF-MS), Real-time PCR

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LIST OF ABBREVIATIONS

- **AASLD**: American Association for the Study of Liver Diseases.
- **ACD:** anemia of chronic diseases
- **AFP:** alpha feto protein
- **ALT**: alanine aminotransaminase.
- **ALP**: alkaline phosphatase.
- **ANA:** anti nuclear antibody.
- **AST**: aspartate aminotransaminase.
- **BMI**: body mass index.
- **BMPs**: Bone morphogenetic proteins
- **CHC**: chronic hepatitis C.
- **Dcytb**: Duodenal cytochrome b
- **DIOS:** dysmetabolic iron overload syndrome
- **DM**: diabetes milletus.
- **DMT-1:** divalent metal transporter 1
- **EASL**: European Association for the Study of the Liver.
- **EDHS:** Egypt Demographic and Health Survey
- **ELISA**: enzyme-linked immunoassay
- **EPO**: erythropoietin
- **ETR**: end of treatment response.
- **EVR**: early virological response.
- **FPN:** ferroportin
- **GDF**-15: growth differentiation factor–15

- **GGT**: gamma glutamyl transpeptidase.
- **HCC**: hepatocellular carcinoma.
- **HBV**: hepatitis B virus.
- **HCV:** hepatitis C virus.
- **HIF**: hypoxia inducible factor
- **HIC**: hepatic iron concentration
- **HII:** hepatic iron index
- **HIV:** human immnuno deficiency virus.
- **HFE:** hemochromatosis protein
- **HJV**: hemojuvelin
- **HPC1:** Heme protein carrier1
- **ID**: iron deficiency
- **IL-6**: Interleukin 6
- **INF**: interferon
- **INR**: international normalized ratio.
- **IRP1:** iron regulatory protein 1.
- **IRP1**: iron regulatory protein 1
- **LDH:** lactate dehydrogenase**.**
- **LEAP**: liver–expressed antimicrobial peptide
- **m-HJV:** Membrane isoform of hemojuvelin
- **m RNA:** messenger ribonucleic acid
- **NF-kB**: nuclear factor kappa-light-chain-enhancer of activated B cells
- **NHIOD**: non hereditary mild iron overloading hepatic disease
- **NRAMP:** resistance macrophage protein

- **PCR**: Polymerase chain reaction.
- **PEG-IFN**: pegaylated interferon.
- **PT**: prothrombin time.
- **8-OHdG**: 8-hydroxy-2′ -deoxyguanosine
- **RBV**: ribavirin.
- **RNA**: ribonucleic acid.
- **ROS**: Reactive oxygen species
- **RVR**: rapid virological response.
- **SEM**: standard error of mean.
- **SCD:** sickle cell disease
- **SGOT**: glutamic oxaloacetic transaminase.
- **SGPT**: glutamic pyruvic transaminases.
- **SMAD**: mothers against decapentaplegic homolog
- **SOCS:** Suppressor of cytokine signaling
- **STAT 3**: signal transducer and activator of transcription 3
- **SVR**: sustained virological response.
- **TBRI:** Theodor Bilharz Research Institute
- **TfR**: Transferrin receptor
- **TLR-4:** Toll-like receptor 4
- **TS**: transferrin saturation
- **TSH**: thyroid stimulating hormone.
- **ULN**: upper limit of normal.
- **vHL**: von Hippel-Lindau
- **WHO**: World Health Organization.

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INTROUDUCTION

Hepcidin was first discovered in human blood ultrafiltrate and urine samples as a small bactericidal peptide (defensin and cathelicidin) and named liver–expressed antimicrobial peptide (LEAP–1) ***(Fleming, 2008).***

The name 'hepcidin' originates from the place of synthesis in hepatocytes (hep-) and its antimicrobial activity (-cidin) ***(Politou et al., 2004).***

The gene encoding hepcidin (HAMP, 19q13) is expressed in the liver, heart, lungs, brain, spinal cord, intestine, stomach, pancreas, adipocytes, skeletal muscles, testis and macrophages ***(Darshan et al., 2009, Bansal et al., 2009).***

This protein is a key regulator of iron level, it decreases the iron absorption from the duodenal enterocytes, iron release from macrophages and its transport across the placenta ***(Guo et al., 2009, Hoppe et al., 2009).***

The main role of hepcidin in iron metabolism was confirmed on animal models and in vitro studies ***(Camscchella & Silvestri, 2008).***

The synthesis of hepcidin in hepatocytes can be regulated by iron overload, inflammatory signals, increased erythropoiesis, hypoxia and anemia ***(Christiancen et al., 2007; Arruda et al., 2009).***

Iron is an essential element for all living organisms, being a requirement in a wide range of metabolic processes including DNA synthesis, oxygen transport, and energy production, but excess iron can be harmful to the organism, in part through the generation of oxygen radicals, and is potentially lethal ***(Ruivard et al., 2009).***

Therefore, iron homeostasis must be tightly regulated in all organisms. Recent work has established the importance of the peptide hormone hepcidin in iron homeostasis as a negative regulator of iron release into the system by duodenal

enterocytes and reticuloendothelial macrophages ***(Camscchella & Silvestri., 2008; Swinkels & Wetzel's., 2008).***

Hepcidin binds to the iron exporter ferroportin, which results in ferroportin internalization and degradation ***(Fujita et al., 2008).***

In addition to its response to iron homeostasis, hepcidin is induced by inflammation ***(Oguz et al., 2006) .***

how hepcidin levels are kept in balance through upstream signaling pathways is still under investigation. An effect believed to be dependent on cytokine production ***(Arruda et al., 2009).***

Patients with chronic hepatitis C (CHC) often have increased liver iron, a condition associated with reduced sustained response to antiviral therapy, more rapid progression to cirrhosis, and development of hepatocellular carcinoma. The hepatic hormone hepcidin is the major regulator of iron metabolism and inhibits iron absorption and recycling from erythrophagocytosis. Hepcidin decrease is a possible pathophysiological mechanism of iron overload in CHC, but studies in humans have been hampered so far by the lack of reliable quantitative assays for the 25-amino acid bioactive peptide in serum (s-hepcidin) ***(Girelli et al., 2009).***

Very recent studies in animals and cellular model have suggested that HCV infection may directly modulates hepcidin expression ***(Nishina et al., 2008*).**

AIM OF THE WORK

- To determine the clinical relevance of hepatic producing iron regulatory hormone-hepcidin, on iron overload in patients with chronic hepatitis C (CHC).
- To detect the effect of interferon based therapy on hepcidin level correlating it with early virological response (This may be later used as a predictor of response to interferon therapy according to hepcidin levels).

Chapter(1)Treatment of CHC

Introduction:

Hepatitis C virus (HCV) infection is one of the main causes of chronic liver disease worldwide ***(Lavanchy ,2009)***. The number of chronically infected persons worldwide may exceed 200 million, but most of them have no knowledge of their infection or of the ensuing hepatic condition***(Antonio,2011).***

The highest HCV prevalence in the world occurs in Egypt .15 percent of the Egypt Demographic and Health Survey (EDHS)respondents aged 15-59 had antibodies to the HCV virus in their blood, indicating that they had been exposed to the virus at some point. 10 percent were found to have an active infection ***(El-Zanaty,2008)***. Considerable regional differences(Fig I)The highest HCV prevalence in the world occurs in Egypt (20%), where the prevalence of infection increases steadily with age, and high rates of infection are observed among persons in all age groups***(Medhat et al.,2002).***

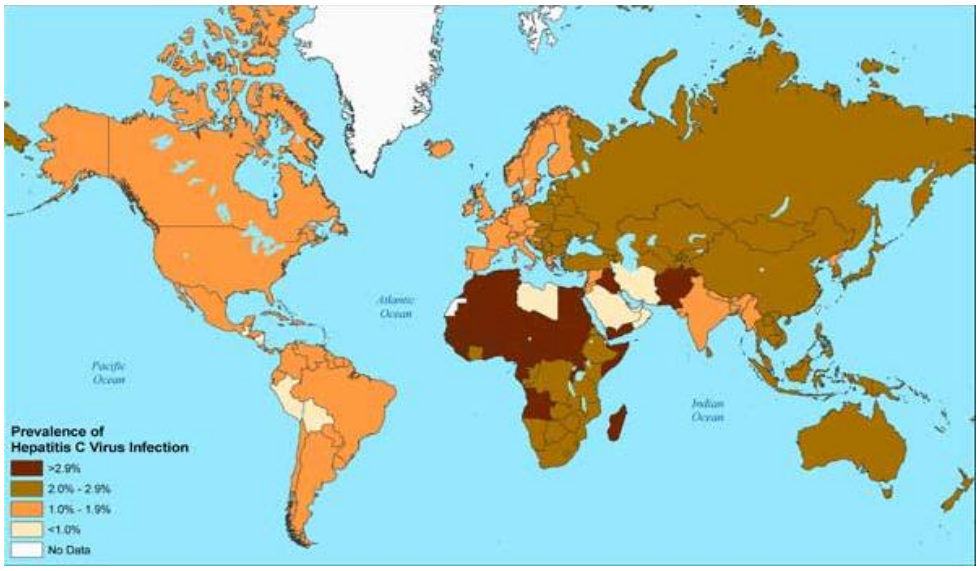

Figure (I): Global prevalence of hepatitis C(Shepard et al., 2005).