Effect of Intra Venous Ascorbic Acid on Hepatic Iron Overload in Prevalent Hemodialysis Patients with Hepatitis C

Thesis

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By

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Mohamed Mostafa Ali

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List of Abbreviations

Abbr.	Full-term
ALT	**:** Alanine aminotransferase
APR	**:** Acute Phase Response
AST	**:** Aspartate aminotransferase
AUC	**:** Area Under the Curve
BMP	**:** Bone morphogenic proteins
Ca	**:** Calcium
CHC	**:** Chronic Hepatitis C
CI	**:** Confidence Interval
CKD	**:** Chronic Kidney Disease
Cp	**:** Ceruloplasmin
CRP	**:** C-reactive protein
CVD	**:** Cardiovascular Disease
Dcytb	**:** Duodenal cytochrome b
DFO	**:** Deferoxamine
DFP	**:** Deferiprone
DMT1	**:** Divalent metal transporter 1
EBP	**:** Enhanced Binding Protein
EPO	**:** Erythropoietin
ESA	**:** Erythropoietin-Stimulating Agents
ESRD	**:** End Stage Renal Disease
Fe	**:** Iron
Fe2+	**:** Ferrous
Fe3+	**:** Ferric
Fpn	**:** Ferroportin
Ft	**:** Ferritin
GPI	**:** Glycosylphosphatidylinositol
HAMP	**:** Hepcidin antimicrobial peptide
Hb	**:** Hemoglobin
HCC	**:** Hepatocellular Carcinoma

HCV : Hepatitis C Virus
Heph : Hephaestin
HFE2 : Hemojuvelin
HIF : Hypoxia Inducible Factor
HIV : Human Immunodeficiency Virus
HJV : Hemojuvelin
IL : Interleukin
iPTH : Intact Parathyroid Hormone
IRE : Iron-responsive Elements
IRMA : Immunoradiometric assay
IRP : Iron regulatory Proteins
IV : Intra-venous
JAK-STAT : Janus-Associated Kinase–Signal Transducers and Activators of Transcription
LIC : Liver Iron Concentration
LPI : Labile Plasma Iron
MHC : Major Histocompatibility Complex
MICS : Malnutrition-Inflammation-Cachexia Syndrome
MRI : Magnetic Resonance Imaging
mRNA : Messenger RNA
NPV : Negative Predictive Value
NTBI : Non–transferrin bound iron
PCR : Polymerase Chain Recation
PO : Serum Phosphorus
PO2 : Partial Pressure of Oxygen
PPV : Positive Predictive Value
PT : Prothrombin Time
PTH : Parathyroid Hormone
Retics : Reticulocyte count
ROC : Receiver operating characteristic
ROI : Region of Interest
ROS : Reactive oxygen species
SD : Standard Deviation
SIR : Signal Intensity Ratio

SQUID : Superconducting Quantum Interference Device
SSE : Single Spin Echo
STAT : Signal Transducer and Activator of Transcription
Tf : Transferrin
TfR1 : Transferrin receptor 1
TfR2 : Transferrin receptor 2
TIBC : Total Iron Binding Capacity
TNF : Tumor Necrosis Factor
TSAT : Transferrin Saturation Ratio
URR : Urea Reduction Ratio

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Introduction

Anemia is common in patients with end-stage renal disease (ESRD) and is a risk factor for hospitalization and mortality. It is mainly due to an absolute or relative decrease in erythropoietin (EPO) production by the failing kidney. Several other factors (iron and vitamin deficiency, infection, inflammation, occult blood loss, oxidative stress, inadequate dialysis, and hyperparathyroidism) often contribute to anemia development and reduce response to treatment ***(Bonomini et al., 2015).***

Administration of IV iron to hemodialysis (HD) patients complements ESA therapy, helps maintain target hemoglobin levels, and lowers ESA dosing requirements ***(Provenzano et al., 2009).***

However, IV iron use requires careful balance between intended clinical effect and uncertain risks of toxicities. Many authors have raised concerns regarding the hazards of IV iron in HD patients ***(Kamanna et al., 2012).***

Recent studies demonstrated that dysregulated iron metabolism is a common feature in HD patients and that it may cause progressive atherosclerotic cardiovascular disease through several pathogenic mechanisms, including oxidative stress induction, an increase in susceptibility to infection,

vascular endothelial damage, and immune dysfunction ***(Nakanishi et al., 2015).***

The liver is the main site of iron storage in humans, and the liver iron concentration (LIC) correlates closely with total body iron stores in healthy persons as well as patients with genetic hemochromatosis and secondary hemosiderosis such as thalassemia major and sickle cell disease. It seems very likely that iron overload in hemodialysis patients follows the same rules. In systemic iron overload, up to 70% to 90% of total body iron stores are found primarily in hepatocytes and Kupffer cells, mainly as ferritin and hemosiderin iron ***(Rostoker et al., 2015).***

Hepatic magnetic resonance imaging (MRI) has now emerged as the gold-standard method for estimating and monitoring iron stores in genetic hemochromatosis and secondary hemosiderosis. Given its accuracy, availability, and relative non-invasiveness, measuring the LIC using MRI seems to be the best indicator of iron overload in hemodialysis patients ***(Rostoker et al., 2015).***

In chronic hepatitis, hepatic iron deposition is found in 35–56% of cases. This was especially demonstrated in patients with chronic hepatitis related to the HCV. Iron excess has been shown to correlate with necro-inflammatory changes and to decrease after antiviral therapy ***(Deugnier &Turlin, 2011).***

Moreover, the liver-produced hormone hepcidin regulates levels and compartmentalization of iron by inhibiting the iron exporter ferroportin. Hepcidin excludes iron from serum by sequestering it in macrophages and preventing dietary uptake ***(Ganz, 2013).***

Vitamin C (ascorbic acid) is a cofactor in numerous metabolic reactions. Dietary ascorbate enhances iron absorption in the gut, and can regulate cellular iron uptake and metabolism. Vitamin C has been reported to increase the release of iron from ferritin and the reticuloendothelial system and increase iron utilization during heme synthesis ***(Lane & Richardson, 2014).***

Furthermore, ascorbate cycling across the plasma membrane is responsible for ascorbate-stimulated iron uptake from iron–citrate complexes, found in the plasma of individuals with iron overload disorders. Importantly, this iron-uptake pathway is of relevance in patients with iron overload (***Lane & Richardson, 2014).***

Various studies showed that in HD patients, vitamin C seems to favor iron mobilization from tissue stores and to increase iron utilization. It was shown that low amount of intra venous ascorbic acid could reduce ferritin level and enhance Hb and TSAT, suggesting improved iron utilization ***(Jalalzadeh et al., 2012).***

Though benefits of IV vitamin C supplementation to HD patients have been reported in various studies, further well-controlled studies are needed before this form of therapy can be generally recommended. In fact, the 2012 KDIGO guidelines for anemia management in CKD acknowledged the potential benefits of ascorbic acid as an adjunct therapy, but stated that there were not enough large randomized trials to recommend vitamin C in the guidelines ***(Locatelli et al., 2013).***

Aim of the Work

To evaluate hepatic iron concentration in prevalent HD patients with hepatitis C virus infection, and to study the efficacy of intravenous ascorbic acid in reducing the hepatic iron overload.

Iron Physiology & Pathophysiology

Introduction

By 1979, it was thought that the basic concepts of iron physiology were understood, including the iron cycle within the body, plasma iron kinetics and exchange with tissues, and storage ***(Anderson & McLaren, 2012)***.

However, recent advances in cellular and molecular levels had reached to the stage that we can now study mechanisms of iron transport by transferrin and the process of iron storage in ferritin and hemosiderin, and in retrospect, it is now apparent that most of the proteins of iron transport and regulation were then unknown ***(Lane et al., 2015)***.

Biochemistry and Physiology of Iron

With minor exceptions, almost all cells employ iron as a cofactor for fundamental biochemical activities, such as oxygen transport, energy metabolism and DNA synthesis. This is due to the flexible coordination chemistry and redox reactivity of iron, which allow it to associate with proteins and bind to oxygen, transfer electrons or mediate catalytic reactions ***(Ebrahimi et al., 2014)***.

However, iron is also potentially toxic, because, under aerobic conditions, it catalyzes the propagation of ROS