Assessing the relationship between hepatitis C virus and porphyria cutanea tarda

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ASSESSING THE RELATIONSHIP BETWEEN HEPATITIS C VIRUS AND PORPHYRIA CUTANEA TARDA

by

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B.A., Boston College, 2011

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ASSESSING THE RELATIONSHIP BETWEEN HEPATITIS C VIRUS
AND PORPHYRIA CUTANEA TARDA

JOHN J. BYUN
Boston University School of Medicine, 2013

Major Professor: Gwynneth Offner, Ph.D., Associate Professor of Medicine

ABSTRACT

Porphyria cutanea tarda (PCT) is the most common form of porphyria, diseases that arise from decreased activity levels of enzymes in the heme biosynthetic pathway. Unlike other porphyrias that are caused by genetic mutations, PCT is often caused by exogenous factors, which include alcohol abuse, human immunodeficiency virus (HIV) and hepatitis C virus (HCV) infection. Many studies have shown that there is an overlap in the population of patients who have PCT and HCV infection. HCV infection, in particular, causes inflammation and fibrosis in the liver, and disrupts overall homeostasis of the body, especially with respect to iron metabolism. Both diseases present symptoms that are either the cause or the effect of iron overloading in the liver. Iron overloading leads to oxidative stress in the body which further propagates symptoms of PCT and HCV. This review will investigate the causes, symptoms, and treatment modalities for patients with PCT and HCV infection. It will also evaluate the risk factors, such as iron overload and oxidative stress, which may contribute to the overlap among patient populations with the two diseases.
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<table>
<thead>
<tr>
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<th>Description</th>
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<tr>
<td>ALA</td>
<td>Aminolevulinic Acid</td>
</tr>
<tr>
<td>ALP</td>
<td>Alkaline Phosphatase</td>
</tr>
<tr>
<td>ALT</td>
<td>Alanine Aminotransferase</td>
</tr>
<tr>
<td>AIP</td>
<td>Acute Intermittent Porphyria</td>
</tr>
<tr>
<td>ADP</td>
<td>ALA-dehydratase- Deficient porphyria</td>
</tr>
<tr>
<td>AST</td>
<td>Aspartate Aminotransferase</td>
</tr>
<tr>
<td>ARE</td>
<td>Antioxidant Response Element</td>
</tr>
<tr>
<td>CD31</td>
<td>Cluster of Differentiation 31</td>
</tr>
<tr>
<td>CEBP</td>
<td>CCAAT/enhancer-binding protein</td>
</tr>
<tr>
<td>CHOP</td>
<td>C/CBP Homology Protein</td>
</tr>
<tr>
<td>CoA</td>
<td>Coenzyme A</td>
</tr>
<tr>
<td>CTGF</td>
<td>Connective Tissue Growth Factor</td>
</tr>
<tr>
<td>DcytB</td>
<td>Duodenal Cytochrome B</td>
</tr>
<tr>
<td>DMT-1</td>
<td>Divalent Metal-ion Transporter 1</td>
</tr>
<tr>
<td>eIF4F</td>
<td>Eukaryotic Initiation Factor 4F</td>
</tr>
<tr>
<td>fPCT</td>
<td>Familial PCT</td>
</tr>
<tr>
<td>FPN1</td>
<td>Ferroportin</td>
</tr>
<tr>
<td>GAG</td>
<td>Glycosaminoglycans</td>
</tr>
<tr>
<td>GGT</td>
<td>Gamma-Glutamyltransferase</td>
</tr>
<tr>
<td>GT</td>
<td>Glutamyl Transpeptidase</td>
</tr>
<tr>
<td>HAV</td>
<td>Hepatitis A Virus</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>Hb</td>
<td>Hemoglobin</td>
</tr>
<tr>
<td>HBV</td>
<td>Hepatitis B Virus</td>
</tr>
<tr>
<td>HCC</td>
<td>Hepatocarcinoma Cells</td>
</tr>
<tr>
<td>HCV</td>
<td>Hepatitis C Virus</td>
</tr>
<tr>
<td>HDAC</td>
<td>Histone Deacetylase</td>
</tr>
<tr>
<td>HDV</td>
<td>Hepatitis D Virus</td>
</tr>
<tr>
<td>HEV</td>
<td>Hepatitis E Virus</td>
</tr>
<tr>
<td>HFE</td>
<td>Human Hemochromatosis Protein</td>
</tr>
<tr>
<td>HIV</td>
<td>Human Immunodeficiency Virus</td>
</tr>
<tr>
<td>HJV</td>
<td>Hemojuvelin</td>
</tr>
<tr>
<td>HSC</td>
<td>Hepatic Stellate Cells</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>IRES</td>
<td>Internal Ribosome Entry Site</td>
</tr>
<tr>
<td>Keap1</td>
<td>Kelch-like Associated Protein 1</td>
</tr>
<tr>
<td>LDL</td>
<td>Low Density Lipoprotein</td>
</tr>
<tr>
<td>LDL-R</td>
<td>Low Density Lipoprotein Receptor</td>
</tr>
<tr>
<td>LFT</td>
<td>Liver Function Test</td>
</tr>
<tr>
<td>MMP</td>
<td>Metalloproteinase</td>
</tr>
<tr>
<td>NAD(P)</td>
<td>Nicotinamide Adenine Dinucleotide (Phosphate)</td>
</tr>
<tr>
<td>NANBH</td>
<td>Non-A Non-B Hepatitis</td>
</tr>
<tr>
<td>Nramp2</td>
<td>Natural Resistance-Associated Macrophage Protein 2</td>
</tr>
<tr>
<td>NS</td>
<td>Nonstructural Proteins</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
</tr>
<tr>
<td>PCT</td>
<td>Porphyria Cutanea Tarda</td>
</tr>
<tr>
<td>R</td>
<td>Relaxed - Hb orientation</td>
</tr>
<tr>
<td>RBC</td>
<td>Red Blood Cells</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive Oxygen Species</td>
</tr>
<tr>
<td>SOD</td>
<td>Superoxide Dismutase</td>
</tr>
<tr>
<td>sPCT</td>
<td>Sporadic PCT</td>
</tr>
<tr>
<td>T</td>
<td>Taut – Hb orientation</td>
</tr>
<tr>
<td>Tfr1</td>
<td>Transferrin Receptor 1</td>
</tr>
<tr>
<td>TGF-β</td>
<td>Transforming Growth Factor-β</td>
</tr>
<tr>
<td>Ub</td>
<td>Ubiquitin</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>VLDL</td>
<td>Very Low Density Lipoprotein</td>
</tr>
<tr>
<td>VP</td>
<td>Variegate Porphyria</td>
</tr>
</tbody>
</table>
Hepatitis

Introduction

The liver, which is located at the junction of the circulatory system and the digestive system, has a vast array of functions. It processes dietary elements which are digested and absorbed in the gastrointestinal tract and synthesizes many important molecules and serum proteins, such as coagulation factors and albumin. The liver also participates in the systemic immune response by synthesizing various innate immune response-related proteins during early stages in immunological response. Another function of the liver is the detoxification and elimination of endogenous and exogenous toxins. The unique blood supply of the liver results in repeated exposure to foreign antigens and toxins so that the liver must continuously distinguish the toxic from non-toxic material. The liver provides an effective barrier and protection for the body against infections that arise from the gastrointestinal tract. Because of these functions, the liver has a major role in assisting in not only in maintenance of metabolic homeostasis, but homeostasis of the body as a whole (Tang et al., 2009).

Inflammation is a multifaceted interaction between various soluble factors in tissues as a response to infection, trauma, autoimmune and toxic injury. While inflammation plays a crucial role in clearing pathogens and healing injured tissues, it can also result in tissue damage and loss of function of the tissue or organ. Inflammation and swelling in the liver is called hepatitis; though Hepatitis is a disease that is marked by the inflammation and swelling of the liver. Although not all hepatitis is caused by viral infections, many are. There are two major classes of hepatitis: non-viral and viral
hepatitis. Non-viral hepatitis is caused by other factors, such as alcohol, toxins and autoimmunity. There are many different types of viral hepatitis with each caused by different types of virus (Hall, 2007).

Non-viral Hepatitis

Non-viral hepatitis is a class of diseases that is marked by inflammation in the liver caused by factors other than viral infection. Autoimmune hepatitis is a non-viral hepatitis that is caused by the immune system of the patient and has two types: type 1 and type 2. Type 1 can occur at any age, and half the patients also have other autoimmune diseases, such as type 1 diabetes and thyroiditis. Type 2 autoimmune hepatitis is the less common form which typically affects girls between ages of 2 and 14, and often occurs with other autoimmune problems (Mayo Clinic, Autoimmune Hepatitis, 2012).

Toxins, alcohol in particular, in the body can also cause non-viral hepatitis. When alcohol is processed in the liver and is broken down, it produces harmful chemicals such as acetaldehyde, which can trigger inflammation responses in the liver. In the inflammation process, healthy hepatocytes are replaced with scars and knots of tissue that causes permanent damage in the liver and cause cirrhosis, or liver fibrosis. Although the risk for alcoholic hepatitis increases with the amount of alcohol consumed, the disease does not necessarily correlate with it. Only 35% of heavy long-term drinkers develop alcoholic hepatitis, and moderate drinkers may be affected by the disease (Mayo Clinic, Alcoholic Hepatitis, 2012). The liver also processes other exogenous substances, such as prescription medications and industrial chemicals, which can also create stress in the liver.
and cause toxic hepatitis. People who either take over-the-counter or prescription drugs or have liver diseases have a higher chance of developing toxic hepatitis. Being female is another risk factor that is common in alcoholic and toxic hepatitis. Since females metabolize toxins more slowly than males do, their bodies are exposed to higher level of harmful substances for longer period of time, leading to increased risk of hepatitis (Mayo Clinic, Toxic Hepatitis, 2010).

**Viral Hepatitis**

There are five types of hepatitis that are caused by viral infection: hepatitis A, B, C, D and E. Viruses are transmitted by the fecal-oral route, perinatally, percutaneously, through blood to blood contact, or unprotected intercourse (Hall, 2007). All of the viruses cause acute hepatitis, and some, such as hepatitis B, C, and D, can cause chronic conditions. Hepatitis A virus (HAV) and Hepatitis E virus (HEV) primarily spread through water or food that is contaminated with feces of an infected person. Therefore, men who have sex with men and international travelers to developing countries are at a higher risk of being infected with the two viruses (National Institute of Health, Viral Hepatitis, 2008). Hepatitis A and E viruses resolve themselves within several weeks to several months. Among patients who have HAV infection, 85% of them will recover within three months and the remaining 15% will recover within the next three months. HEV has an incubation period of 15-60 days and has a mortality rate of 15-25% among pregnant women. Most others who are infected with the virus recover within six weeks.
after the incubation period (Hall, 2007). Unlike HAV, there is no vaccine for HEV (National Institute of Health, Viral Hepatitis, 2008).

Hepatitis B virus poses a great challenge to the global health care system because of the high number of people who are infected with the disease. It is estimated that over 350 million people are infected with hepatitis B virus (HBV), which can be transmitted through blood to blood contact or unprotected sexual intercourse (Hall, 2007). It has an incubation period of two to six weeks, after which patients enter the acute phase. 3-5% of adults and 95% of children who cannot generate enough immunological response then develop a chronic condition (Hall, 2007). Patients with chronic HBV infection may develop cirrhosis, hepatic decompensation, and hepatocellular carcinoma, which is liver cancer. Hepatitis D virus (HDV) is similar to HBV since HDV spreads through contact with infected blood and unprotected sexual contact. However, HDV can only infect patients who have concurrent HBV infection. Injection drug users and those who have received blood transfusion before 1987 are among those who have the highest risk. Since there is no vaccine for HDV, one of the best ways to prevent HDV infection is to avoid risk factors, such as using contaminated needles and razors. Since only those with HBV infection can be infected with HDV and there is a vaccine for the former, those who do not have HBV infection should receive vaccination (National Health Institute, Viral Hepatitis, 2008).
**Hepatitis C Virus**

The last of the viral hepatitis is called hepatitis C, which is caused by hepatitis C virus. It was first discovered in 1975 in post-transfusion hepatitis patients, and was named NANBH, or non-A non-B hepatitis. Like HBV infection, HCV infection and its associated liver complications have become a major challenge in the global health system. Since its discovery, the number of infections has grown steadily and it is estimated that 170 million worldwide has chronic HCV infection, which is about 3% of the world’s population; approximately 3.2 million people in the US have chronic HCV infection and 17,000 new HCV cases each year in the US (Center for Disease Control and Prevention, Hepatitis C, 2012).

Much research has been done on HCV and it is now known that the disease is genetically heterogeneous with six major genotypes, and each genotype has varying prevalence in different geographic regions (Simmonds et al., 1992). The following list pairs a genotype and the region where it is the most prevalent.

1a- North and South America
1b- South America, Europe and Asia
2- Europe, South Africa and Japan
3- Australia, South Asia and South America
4- Egypt and Middle East
5- South Africa and Southeast Asia
6- Southeast Asia
7, 8, 9- Southeast Asia (Zein, 2000)
HCV is transmitted both parenterally and perinatally, and is cleared spontaneously in 20% of the cases, with the other 80% developing chronic hepatitis C infection. About 20% of the chronically infected patients eventually sustain permanent liver damage and fibrosis, and 4% develop hepatocellular carcinoma (Schuppan et al., 2003). Currently there is no vaccine for HCV, and its associated complications are the leading cause of liver transplantation in the US and Europe (Wald, 2007).

A positive strand RNA virus of the Flaviviridae family, HCV contains a single-stranded RNA genome that has approximately 9600 nucleotides. This RNA is a template for both mRNA transcription and RNA replication (Tang, 2000). HCV RNA encodes a single protein, which is then processed to give rise to ten structural and nonstructural proteins. Nonstructural proteins, which are named p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B, form an HCV replicase that is located on the membrane of endoplasmic reticulum. Structural proteins, such as core, E1, and E2 form the viral particles (Jeong, 2012).

**Diagnosis**

The range of the incubation period of HCV is 3-20 weeks with the mean period being seven weeks, and antibodies can be detected as early as a week after the infection (Center for Disease Control, ABC’s of hepatitis, 2012). The infection and disease progress can be divided into acute and chronic phases. The former refers to period of time immediately after infection and can last from 3-24 weeks and usually less than six
months. The majority of the patients with HCV infection develop a chronic condition as they are not able to eliminate the virus spontaneously.

There are many ways to evaluate whether or not a patient has been infected with HCV. The first method is to look carefully into the health history and determine the presence of risk factors. People who may be at risk for contracting hepatitis C are those who have been on long-term kidney dialysis or have received blood, blood products or organs from a donor who has hepatitis C. Sharing needles with others who have hepatitis C is a big risk factor as well. It is debated whether or not having unprotected sexual contact with those with hepatitis C is another risk factor as those sexual contacts may be a result of other risk factors. Babies who are born to mothers with the disease are at a higher risk as well (National Institute of Health, Viral Hepatitis, 2008).

One of the tests that can be used to screen for liver disorders is the Liver Function Test (LFT). LFT evaluates the concentrations of liver alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), and alkaline phosphatase (ALP). The normal values of these enzymes in males are as follows: ALT (7-55 u/L), AST (8-48 u/L), GGT (9-48 u/L), ALP (45-115 u/L), albumin (3.5-5.0 g/dL), and total protein (6.3-7.9g/dL) (Mayo Clinic, Live Function Test, 2012).

There are two major classes of assays that are used to diagnose HCV infection. One is a serologic assay that identifies antibodies that are specific to HCV (anti-HCV) in the serum or plasma. The other is a molecular assay that directly detects HCV RNA, and polymerase chain reaction (PCR) has been incorporated recently to these assays to increase the sensitivity of the tests (McOmish et al., 1994; Ghany et al., 2009).
The diagnosis of HCV usually requires a positive test for both anti-HCV antibodies and HCV RNA. One of the goals of the diagnosis process is to differentiate between acute and chronic HCV, and different diagnosis is made depending on the results of the two tests as shown in table 1 (Ghany, 2009).

**Table 1 – Interpretation of anti-HCV and HCV RNA diagnostic exams** (Table amended from Ghany et al. 2009)

<table>
<thead>
<tr>
<th>Anti-HCV</th>
<th>HCV RNA</th>
<th>Interpretation</th>
</tr>
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<tbody>
<tr>
<td>Positive</td>
<td>Positive</td>
<td>Acute or chronic HCV depending on the clinical context</td>
</tr>
<tr>
<td>Positive</td>
<td>Negative</td>
<td>Resolution of HCV; acute HCV during period of low-level viremia</td>
</tr>
<tr>
<td>Negative</td>
<td>Positive</td>
<td>Early acute HCV infections; chronic HCV in setting of immunosuppressed state; false positive HCV RNA test</td>
</tr>
<tr>
<td>Negative</td>
<td>Negative</td>
<td>Absence of HCV infection</td>
</tr>
</tbody>
</table>

Liver biopsy is a useful tool in monitoring chronic HCV progression. It provides information on the extent of the liver injury and if the patient needs to be aware of the risk of developing HCC. The biopsy is given a grade using one of the common staging systems used for evaluating the fibrosis or cirrhosis in liver which is shown in table 2 (Kleiner et al., 2005). Although the staging systems differ from one another, cirrhosis is the last stage, during which liver damage can cause other serious complications.
Table 2 – Comparison of Commonly Used Staging Systems for Chronic Hepatitis C
(Table amended from Kleiner et al., 2005)

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>0: No fibrosis</td>
<td>0: None</td>
<td>0: No fibrosis</td>
<td>0: No fibrosis</td>
<td>0: No fibrosis</td>
</tr>
<tr>
<td>1: Fibrous portal expansion</td>
<td>1: Enlarged, fibrotic portal tracts</td>
<td>1: Stellate enlargement of portal tracts without septae formation</td>
<td>1: Portal fibrosis</td>
<td>1: Fibrous expansion of some portal areas, with or without short fibrous septa</td>
</tr>
<tr>
<td>2: Periportal fibrosis</td>
<td>2: Periportal or portal-portal septa but intact architecture</td>
<td>2: Enlargement of portal tracts with rare septae formation</td>
<td>2: Periportal fibrosis</td>
<td>2: Fibrous expansion of most portal areas, with or without short septa</td>
</tr>
<tr>
<td>3: Bridging fibrosis (portal-portal or portal-central linkage)</td>
<td>3: Fibrosis with architectural distortion but no obvious cirrhosis</td>
<td>3: Numerous septae without cirrhosis</td>
<td>3: Septal fibrosis</td>
<td>3: Fibrous expansion of most portal areas with occasional portal to portal bridging</td>
</tr>
<tr>
<td>4: Cirrhosis</td>
<td>4: Probable or definite cirrhosis</td>
<td>4: Cirrhosis</td>
<td>4: Cirrhosis</td>
<td>4: Cirrhosis</td>
</tr>
</tbody>
</table>

Symptoms

Most people who are recently infected with HCV do not show any symptoms, but only about 10-20% of them develop jaundice and other nonspecific symptoms such as
fatigue and nausea (Caballes et al., 2012). About 80% of patients who contract HCV develop a long-term, or chronic, infection (Hall, 2007). Although there are no symptoms in early stages, if the infection is present for many years, the liver may develop inflammation which then leads to fibrosis in the liver, which is also called cirrhosis. Some of the tissue may be damaged due to HCV, but persistent inflammation leads to proliferation of fibroblasts and epithelial cells. This may lead to loss of function in these tissues, leading to decreased overall efficiency of the liver. Some symptoms that result from loss of liver tissues are abdominal pain, abdominal swelling, fatigue, fever and vomiting. In some cases, red blood cells and heme cannot be broken down and excreted normally, leading to the accumulation of bilirubin in the body and jaundice, or yellowing of skin (National Institute of Health, Hepatitis C, 2011).

Inflammation and proliferation of certain cells may lead to selection of cells that are at higher risk of malignant transformation. In 4% of the cases, this process leads to the development of hepatocellular carcinoma (Wald et al., 2007). There are a few HCV proteins that have been shown to change the regulation of the cellular proliferation process. HCV core proteins, which are coded by the C region of the HCV genome, use many different mechanisms to induce oncogenesis, such as inhibition of apoptosis and stimulation of growth factors and their respective receptors. They also activate transcription factors/proto-oncogenes and alter the tumor suppressor gene p53. NS(nonstructural protein)3 and NS5A interact with it to lower the rate of apoptosis.

HCV also attacks another tumor suppressor gene named pRb. Two specific proteins, core and NS5B in particular, have been shown to reduce the concentration of
pRb in liver cells. NS5B directly interacts with the tumor suppressor to attract E3 ubiquitin ligase E6AP (E6-associated protein), which degrades pRb. This then contributes to uncontrolled proliferation of cells and cancer (Jeong et al., 2012).

*Life cycle of HCV*

HCV infects the liver *in vivo*, and it uses certain attachment and entry factors to cross the hepatocyte cell membrane and to release its RNA in the cell. The HCV genome is a single stranded RNA with 9600 nucleotides which acts as a template for RNA replication and translation. The polyprotein which is synthesized by translation is modified both co-translationally and post-translationally. Processed viral polyprotein is assembled into new virions and is released by the host cell.

Although the exact mechanism for HCV assembly is still being researched, most agree that HCV replication takes place in the host cell, and the virus is exocytosed after it is made into a complex with VLDLs (Very Low Density Lipoprotein) in chronic HCV patients (Tang et al., 2009). As shown in figure 2, it is also thought that NSs are divided into two pools in the infected cells. One participates in the replication process and is incorporated into the RC, whereas the other is involved in particle assembly (Tang et al., 2009).
HCV utilizes receptor mediated endocytosis to enter host cells, and this requires coordination of different receptors such as LDL-R (low density lipoprotein receptor), GAG (glycosaminoglycans or heparin sulfate) and CD81. Once the initial interaction is established between HCV and GAG and LDL-R, CD31 binds to the virus to allow entry into the cell as demonstrated in figure 1 (National Institute of Health, 2006).

After HCV enters the cell, it undergoes translation and RNA replication to infect the cell and create new virions. Eukaryotic translation is normally initiated when the mRNA 5’-cap recruits eIF4F (eukaryotic initiation factor 4F) and the 40 S ribosomal subunit binds to the 5’ end and scans for a start codon. Since the HCV RNA does not contain a 5’-cap, it must use an alternate way to initiate protein translation in an IRES (internal ribosome entry site)-based cap-independent approach. RNA replication takes
place in a replication complex, where NS’s are anchored to intracellular membranes, using RNA replicase.

![Diagram of HCV entry](image)

**Figure 2 – Entry of HCV into the Target Cell Initiates the Infection Process** (Figure taken from Tang et al., 2009)

**Prevention and treatment**

There is no vaccine for hepatitis C, so the only way to prevent infection is to avoid the risk factors. The goal of treatments used for HCV is to reduce or eliminate virus from the circulatory system in order to slow the progression of the disease. This means to reduce the risk of cirrhosis and complications that may result from this, such as lowered liver functions and development of HCC. Many patients are given antiviral medications such as pegylated interferon alpha and ribavirin; the former is injected
weekly and the latter is taken orally twice a day. Each genotype responds differently to treatments, and patients with genotype 1 are given newer drugs such as telaprevir and boceprevir because it is the least responsive to medications (Zeuzum et al., 2008; National Institute of Health. Hepatitis C, 2011; Naggie, 2012). In patients that have liver fibrosis, the chance of development of hepatocellular carcinoma may be reduced by a fifth if they respond well to anti-HCV interferon therapy (Tang et al., 2009). If patients develop cirrhosis or hepatocellular carcinoma, they may require a liver transplant.

Porphyria

Introduction

The circulatory system is present in every organism, and its main function is to transport nutrients and waste. In humans, the circulatory system transports oxygen from the lungs to all the tissues of the body, and carbon dioxide from the tissues to the lungs. Since oxygen is not highly soluble in water and dissolved oxygen cannot meet the demands of human body, it utilizes a certain type of hemoprotein to carry oxygen (National Institute of Health. Oxygen transport, 2011).

All hemoproteins contain a heme molecule, which is ubiquitous in all living organisms, and an apoprotein. Heme is synthesized from simple molecules such as succinyl-Coenzyme A (CoA) and glycine. An enzyme called aminolevulinic acid synthetase combines the two compounds to make aminolevulinic acid (ALA), and eight ALAs are used to make protein the porphyrin part of the heme. On the last step of the heme biosynthetic pathway, iron ion is fixed on the four nitrogen atoms that are located at
the center of the heme (Champe, 2008). Heme is poorly soluble in water so it is associated with apoproteins, which then forms hemoproteins. There are many types of hemoproteins such as myoglobins, hemoglobin, and cytochrome c, b, a. (Li et al., 2011)

Heme has many functions in transportation and storage of diatomic gas and electron transfer in myoglobin, hemoglobin and cytochrome c. There are several different types of heme but heme b and heme c are the two most common forms, which are shown in figure 3. Heme b interacts with apoprotein noncovalently and is used in hemoglobin and myoglobin. Heme c interacts with apoprotein covalently between heme vinyl and cysteine residues of the apoprotein, and is used in the cytochrome c complex. The function of the heme protein is affected by many variables such as the microenvironment of the proteins and solvent accessibility to heme (Li et al., 2011).

Figure 3 – Structures of Heme B and Heme C. Heme b interacts with apoproteins noncovalently whereas heme c covalently. Heme c forms a covalent bond with the sulfur group on cysteine (Figure taken from Li et al., 2011)
The human circulatory system utilizes hemoglobin (Hb), one of the heme b hemoproteins, to carry oxygen from the lungs to the tissues. The normal range of Hb in males is 13.8 to 17.2 g/dL and in females 12.1 to 15.1 g/dL (Medline Plus, 2013). Hb has two pairs of subunits named alpha and beta chains. Alpha and beta chains have two forms which are taut (T) and relaxed (R); the former refers to the configuration of hemoglobin when it has oxygen to it and the latter when it is not. Hb has the unique property where all four subunits interact with one another; when one binds to an oxygen molecule, the others are more likely to bind an oxygen molecule as well. Equally, when one of the subunits drop off the oxygen molecule, the others are more likely to do the same. This is often referred to as ‘cooperative binding’ (Freeman, 2008).

Figure 4 – The saturation curve of Hb under standard conditions T=37°C, pH= 7.4 and $P_{O_2} = 40\text{mm Hg}$ (Figure taken from National Institute of Health at http://www.ncbi.nlm.nih.gov/books/NBK54103/)
Cooperative binding induces an allosteric effect where relationship between the partial pressure of oxygen and percent saturation of Hb is a sigmoid curve. At low $P_{O_2}$ or high $P_{O_2}$ the curve has a small slope whereas the middle of the saturation curve between 20-80% of the curve is much steeper. This unusual cooperative binding maximizes the ability of Hb to bind to molecular oxygen at high partial pressure and unbind at low partial pressure. Figure 4 is the saturation curve of Hb at standard physiological conditions under which body temperature is 37°C, pH is 7.4 and $P_{CO_2}$ is 40mm Hg (NIH, oxygen transport, 2011).

The following equation describes the Hb oxygen saturation curve, where $S_{O_2}$ gives the fraction of the Hb that is bound to oxygen:

$$
S_{O_2} = \frac{(P_{O_2}/P_{50})^n}{1 + (P_{O_2}/P_{50})^n}
$$

The term $n$ is Hill’s coefficient, which is 2.7 for human adult Hb, and $P_{50}$ is the partial pressure of oxygen at which half the Hb is saturated.

Hb saturation curve has other variables that can shift it such as temperature, pH, $P_{CO_2}$ and 2, 3-DPG. The affinity of Hb for oxygen gets lower as the curve moves to the right and high as it moves to the left (NIH, oxygen transport, 2011), and these changes mainly take place to compensate for the changes that impacts the variables. For example, a person’s temperature will increase and pH decrease when a person is exercising intensely. This means that the body is in need for more oxygen, and the affinity of Hb for oxygen will fall under these conditions. Increase in temperature and decrease in pH
shifts the Hb oxygen affinity curve to the right as it is shown in figure 5 and 6, and will help Hb deliver oxygen more efficiently to the tissues.

Figure 5 – The effect temperature on the saturation curve of Hb.
(Figure taken from National Institute of Health at http://www.ncbi.nlm.nih.gov/books/NBK54103/)

Figure 6 – The effect of pH on Hb saturation curve.
(Figure taken from National Institute of Health at http://www.ncbi.nlm.nih.gov/books/NBK54103/)
Classification of porphyrias

There are eight steps in the heme biosynthetic pathway and eight enzymes to carry out each step. Porphyria refers to a group of eight diseases that can arise in individuals with genetic disposition that causes mutations in the enzymes or risk factors that reduce the level of enzyme activity. The heme biosynthetic pathway regulates itself, so the reduction of enzyme activity in any particular step leads to deregulation of the pathway and overproduction of heme precursors (Lambrecht et al., 2007). Figure 7 shows the eight enzymes in the heme biosynthesis pathway, and corresponding porphyrias that arise when activity level of any one them is lowered.

![Diagram of heme biosynthesis pathway and porphyrias classification]

**Figure 7 – Pathway of heme biosynthesis and classification of porphyria based on reduced activity level or deficiencies present in each enzyme** (Figure taken from Frank et al., 2010)
Different standards may be used to classify porphyrias. One of the common ways is to group them by the site of overproduction of heme precursors. By this criterion, porphyrias can be divided into either hepatic porphyria, which results because of porphyrin precursors made in the liver, or erythropoietic, in which excess porphyrin production occurs in bone marrow erythroid cells (Balwani et al., 2012). Porphyrias can also be separated by the symptoms that it presents. Cutaneous porphyrias can cause accumulation of porphyrin in the skin, hepatocytes, and red blood cells. These accumulations can cause pathophysiological symptoms that are mostly confined to the skin where sunlight will cause built up porphyrins in the skin to react, leading to blisters and scars. Acute porphyrias, on the other hand, affect different parts of the body from cutaneous porphyrias. Their symptoms include abdominal pain, weakness, dementia, and hallucinations. Most patients who have porphyria exhibit symptoms that is typical to either cutaneous or acute porphyrias, but some who have hereditary coproporphyria (HCP) and variegate porphyria (VP) may present both (American Porphyria Foundation, 2010). Acute hepatic porphyrias refer to autosomal dominant porphyrias such as Acute Intermittent Porphyria (AIP), HCP, VP and autosomal recessive ALA-dehydratase-deficient porphyria (ADP).

Porphyrias are rare diseases that affect a small percentage of the population. It is estimated that less than 200,000 people in the US suffer from porphyria, which is about 1 in 1,500. In Europe, the most common type of porphyria, porphyria cutanea tarda, affects about 1 in 10,000 people and the most common acute porphyria, AIP, 1 in 20,000 (American Porphyria Foundation, 2010).
Porphyria Cutanea Tarda

The most common form of porphyria is called Porphyria Cutanea Tarda (PCT). PCT is a cutaneous porphyria that is caused by defect or lowered activity level of uroporphyrinogen decarboxylase (UROD) (American Porphyria Foundation, 2010). UROD is the enzyme that is responsible for the conversion of uroporphyrinogen III to coproporphyrinogen III by decarboxylation of four carbon dioxide molecules (Phillips et al., 2001). PCT is a complex disease which has multigenetic predisposition, and environmental risk factors are necessary for clinical symptoms to surface. The risk factors that can trigger PCT are HCV and human immunodeficiency virus (HIV) infections, alcohol abuse, estrogen therapy and mutations in hemochromatosis (HFE) genotypes (Frank et al., 2010). Symptoms develop when residual and hepatic UROD decreases below a threshold of 25%.

The symptoms manifest themselves on mainly the skin, where photoreactive porphyrins can lead to blistering and scarring. Other symptoms include hepatic accumulation and urinary excretion of uroporphyrins, altered iron indices, and hepatic iron overload (Panton et al, 2013).

PCT is categorized into two subtypes: hereditary PCT (fPCT) and sporadic PCT (sPCT). About 25% of total PCT patients have a genetic disposition that causes mutations in UROD, which then causes the reduced activity level of fPCT. This alone is usually not enough but must be accompanied by external risk factors for clinical symptoms to manifest. The majority of the remaining 75% of PCT patients present with
the sporadic form of the disease. They do not have mutations in UROD locus, and the pathogenesis of the disease is multifactorial (Aarsand et al., 2009).

**fPCT**

fPCT is caused by mutations in UROD gene which is an autosomal dominant disease with low penetrance. UROD cDNA was discovered in 1986 to have an ORF (open reading frame) of 1,104 nucleotides which is a template for polypeptide that is 367 amino acids long (Roméo et al., 1986). This enzyme has a homodimeric structure and has one active site where the carboxylic groups on uroporphyrinogen III are removed to convert it to coproporphyrinogen III (Roméo et al., 1986). There are currently 30 mutations on UROD locus that has been reported and they are listed in table 3 along with the hypothesized effect of the mutation on UROD structure. 20 of them are missense mutation, and the other 10 are mutations that are either nonsense or cause alternative splicing site, and the latter effect tends to be more detrimental than the former (Phillips, 2001).
<table>
<thead>
<tr>
<th>Residue Change</th>
<th>Structural location</th>
<th>Probable effect of mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>G25E</td>
<td>H1-S2</td>
<td>Tight turn, restricted main chain</td>
</tr>
<tr>
<td>P62L</td>
<td>HC-H1</td>
<td>Residue within disordered loop, unknown function or effect of mutation</td>
</tr>
<tr>
<td>A80S/G</td>
<td>S2</td>
<td>No explanation</td>
</tr>
<tr>
<td>V134Q</td>
<td>H2</td>
<td>Polar residue into a hydrophobic pocket</td>
</tr>
<tr>
<td>R144P</td>
<td>H2</td>
<td>Salt bridge between R144 and Q63, helix-helix stability; P is also a helix breaker</td>
</tr>
<tr>
<td>G156D</td>
<td>S3-HG</td>
<td>Tight turn and restricted side-chain access</td>
</tr>
<tr>
<td>M165R</td>
<td>HG</td>
<td>Charged residue inserted into a hydrophobic pocket</td>
</tr>
<tr>
<td>E167K†</td>
<td>HG</td>
<td>Charge reversal and the E167 links residues in a surface loop</td>
</tr>
<tr>
<td>G168R</td>
<td>HG-HH</td>
<td>Tight turn, restricted main chain</td>
</tr>
<tr>
<td>R193P</td>
<td>H3</td>
<td>Pro insertion into middle of helix</td>
</tr>
<tr>
<td>L195F</td>
<td>H3</td>
<td>No explanation</td>
</tr>
<tr>
<td>L216Q</td>
<td>S4</td>
<td>Hydrophilic residue placed into hydrophobic core, near active site</td>
</tr>
<tr>
<td>E218K</td>
<td>S4</td>
<td>Charge reversal and longer side chain, near active site</td>
</tr>
<tr>
<td>H220P†</td>
<td>S4-HI</td>
<td>Histidine links residues in the intersection of several loops, proline is rigid and hydrophobic</td>
</tr>
<tr>
<td>F229L†</td>
<td>H4</td>
<td>No explanation</td>
</tr>
<tr>
<td>F232L†</td>
<td>H4</td>
<td>No explanation</td>
</tr>
<tr>
<td>L253Q</td>
<td>H4-S5</td>
<td>No explanation</td>
</tr>
<tr>
<td>L260T</td>
<td>S5</td>
<td>No explanation, water molecule replaces extra carbon atom</td>
</tr>
<tr>
<td>G281E/V</td>
<td>S6</td>
<td>Mutant side chain protrudes into active site cleft</td>
</tr>
<tr>
<td>L282R†</td>
<td>S6</td>
<td>Charged residue inserted into a hydrophobic pocket, near active site</td>
</tr>
<tr>
<td>R292G†</td>
<td>H6</td>
<td>Arginine forms a salt bridge between H6 and S8</td>
</tr>
<tr>
<td>G303S</td>
<td>S7</td>
<td>Gly in conformationally strained conformation, side-chain conflicts</td>
</tr>
<tr>
<td>N304K†</td>
<td>S2</td>
<td>Tightly packed between H6 and H7, no room for longer Lys chain</td>
</tr>
<tr>
<td>Y311C†</td>
<td>HJ</td>
<td>Tyrosine packs against Arg179 side chain not supported by Cys, at dimer interface</td>
</tr>
<tr>
<td>G318R†</td>
<td>H7</td>
<td>Glycine lies on an internal turn of helix, large side chains disrupt packing</td>
</tr>
<tr>
<td>M324T†</td>
<td>H7</td>
<td>Side chain points toward protein disrupts packing of helix, dimer interface</td>
</tr>
<tr>
<td>R332H</td>
<td>S8</td>
<td>No explanation</td>
</tr>
<tr>
<td>I334T</td>
<td>S8</td>
<td>No explanation</td>
</tr>
</tbody>
</table>

*On the dimer interface.
†Within 0.7 to 1.2 nm of the dimer interface.
sPCT

For patients with sPCT, UROD activity is reduced only in the hepatocytes because it is an acquired disease. Thus far, no UROD mutations have been detected in sPCT patients. Since patients with fPCT and sPCT present the same clinical symptoms with the same risk factors, it is necessary to use other methods to distinguish them. One of the most common strategies used to distinguish the two subtypes of PCT is to measure the UROD activity level in erythrocytes (Balwani et al., 2012). Another method that is used is to measure the level of isocoproporphyrin, which also accumulates along with uroporphyrinogen III in sPCT patients (Tarwater et al., 2008).

Symptoms and Diagnosis

Like other cutaneous porphyrias, the symptoms of PCT are mostly confined to the skin. Photosensitive porphyrins accumulate in the skin, and they can be exposed to light in the UV range, which excites the molecules. Porphyrins reach a high energy state and release the excess energy as fluorescence by forming single oxygen as well as other species that causes damage to the skin in the form of blisters and scars. (Tarwater et al., 2008) Increased hair growth as well as darkening and thickening of the skin are other common symptoms of PCT, but neurological or abdominal complications are not usually associated with the disease. Mild liver abnormalities are common among PCT patients. However, in rare cases, PCT may lead to more serious complications such as cirrhosis and cause permanent liver damage (Lee et al., 2010). It has been noted that many PCT patients have HCV infection, although the reverse does not hold true.
One commonly used exam to screen for PCT is to measure the total porphyrin levels in the plasma. This test is sensitive and specific for cutaneous porphyrias, and also helps to differentiate PCT from Variegate Porphyria (VP), which arises when protoporphyrinogen oxidase is either defective or exhibits lowered activity levels. Along with this test, investigating the patterns of porphyrins in wastes may confirm the diagnosis. Urine has mostly uroporphyrin and 7-carboxylate porphyrins, and feces contain isocopropporphyrin. As it is demonstrated in table 4, increases in porphyria cutanea tarda can increase the uroporphyrin, coproporphyrin and protoporphyrin levels in urine, stool, erythrocytes and plasma (Tarwater, 2008).
Table 4 – Characteristic level of biochemical substances present in urine stool and blood in the presence of PCT and hepatoerythropoietic porphyria (Table amended from Frank et al., 2010)

+ = slightly increased; ++ = moderately increased; +++ = highly increased; ++++ = very increased

<table>
<thead>
<tr>
<th></th>
<th>uroporphyrin</th>
<th>coproporphyrin</th>
<th>protoporphyrin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Urine</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Porphyria Cutanea Tarda</td>
<td>++++</td>
<td>++</td>
<td>normal</td>
</tr>
<tr>
<td>hepatoerythropoietic porphyria</td>
<td>++++</td>
<td>Isocopropo</td>
<td>normal</td>
</tr>
<tr>
<td><strong>Stool</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Porphyria Cutanea Tarda</td>
<td>++</td>
<td>Isocopropo</td>
<td>+++</td>
</tr>
<tr>
<td>hepatoerythropoietic porphyria</td>
<td>normal</td>
<td>Isocopropo</td>
<td>normal</td>
</tr>
<tr>
<td><strong>Blood/erythrocytes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Porphyria Cutanea Tarda</td>
<td>normal</td>
<td>normal</td>
<td>normal</td>
</tr>
<tr>
<td>hepatoerythropoietic porphyria</td>
<td>normal</td>
<td>+</td>
<td>++++</td>
</tr>
<tr>
<td><strong>Blood/plasma</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Porphyria Cutanea Tarda</td>
<td>++</td>
<td></td>
<td></td>
</tr>
<tr>
<td>hepatoerythropoietic porphyria</td>
<td>++</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Treatment*

Treatment for PCT is targeted to different facets of the disease. In order to prevent skin damage, protective clothing and sunscreen should be worn to minimize exposure to sunlight. Since PCT is caused in large part because of risk factors, patients should avoid them by discontinuing any estrogen therapy as well as alcohol use. There are many protocols for phlebotomy, but one is to remove 300ml every week or 500ml
blood every two weeks over a three to six months period. Although this resolves certain skin problems within several months, it can also induce anemia which should be avoided.

Chloroquine is hypothesized reduce the photosensitivity of skin by inhibiting biosynthesis of porphyrins and increasing the rate of their secretion (Frank et al., 2010). Therefore, chloroquine is often used in PCT therapy and remission can be expected after 6-9 months of a 125mg dose twice a week (Frank et al., 2010).

**Iron and Reactive Oxygen Species**

The relationship between PCT and HCV has not been definitively established, but there are evidences that support strong relationship between the two diseases (Gisbert et al., 2003). Since HCV is a more widespread disease than PCT, the percentage of HCV patients who suffer from PCT is significantly lower than that of PCT patients who suffer from HCV. The majority of HCV patients do not suffer from PCT, but it has been shown that about 47% to 50% of PCT patients suffer from HCV (Gisbert et al., 2003). It is thought that HCV leads to fibrosis in the liver and iron overload. Iron generates reactive oxygen species (ROS) which can damage tissues and organs.

**Iron metabolism**

Iron makes up 32% of the total mass of the earth (Von Drygalski et al., 2012), and has many important functions in mammals as it helps Hb deliver oxygen from the lungs to the tissues. Because of its highly reactive property that can damage cells and tissues, iron absorption and transport must be tightly regulated. Iron balance is achieved mainly
by absorption because mammals cannot regulate iron excretion. A normal adult has 3-4 g of iron distributed and stored in several different places. Approximately 3mg of iron is stored in transferrin pool and 300mg in the myoglobin pool (Von Drygalski et al., 2012). The tissue pool, in which liver plays a large role, stores about 200mg in females and 1g in males. 2000mg of iron is bound to heme in red blood cells and is used to carry oxygen.

Red blood cells (RBC) in humans have an average life span of 120 days, so approximately 1% of the total amount must be replaced daily. An average adult has about 1500 to 2000mL of RBC, which means 15mL to 20mL must be turned over each day; each milliliter of RBC contains 1mg of iron, so the body needs 15-20mg of elemental iron to replace the RBC’s. The majority of the iron that is present in plasma and used in heme biosynthesis is derived from the breakdown of erythrocytes in macrophages, but some is absorbed through diet (Anderson et al., 2012).

Iron recycling process

Erythrocytes are taken up and processed by resident macrophages in the liver and the spleen. Once endocytosed by macrophages, RBC’s in erythrocytes are break down the hemoglobin into globin and heme moieties. The former part is further broken down to individual amino acids. The iron in heme is dissociated and is exported through ferroportin. It then forms a complex with transferrin to be transported to the bone marrow to be recycled. This recycling process is very efficient and close to 80-90% of iron is reused in heme biosynthetic pathway. The other 10-20% of iron is stored in the macrophage as ferritin (Von Drygalski et al., 2012).
**Iron absorption in diet**

Iron that is obtained through diet must be solublized before it can be absorbed by intestinal epithelial cells, which are also called enterocytes (Fuqua et al., 2012). The low pH in the stomach achieves this and keeps the iron in the ferric (Fe\(^{+3}\)) state so that it can be absorbed in the proximal small intestine. At the surface of the absorptive cells, the iron encounters DcytB (duodenal cytochrome B) and other reductases, which reduce the ferric to ferrous (Fe\(^{+2}\)) state. DMT-1 (divalent metal-ion transporter 1, which is also named Nramp2 [natural resistance-associated macrophage protein family 2]) carries the ferrous iron into the enterocyte. Although the mechanism for the absorption of heme iron is not understood, it is generally thought that it is absorbed about 5 to 10 times better than elemental iron. Once it is in the cell, it follows one of two paths depending on the iron concentration in the body. If the concentration is high, iron forms a complex with apoferritin to make ferritin, which is then stored in the cell. If the concentration is low, it is transported to the basolateral membrane of the enterocyte to be carried out by ferroportin (Fpn1), which is the only known iron export protein in mammalian cells. Enterocytes that are further down in the gastrointestinal tract have decreasing amount of ferroportin (Anderson et al., 2009).

Once ferrous iron is in the circulation, it undergoes another change in oxidation state by ferroxidase from the ferrous (Fe\(^{+2}\)) to ferric state (Fe\(^{+3}\)) as shown in figure 8. Iron in the ferric state is required in order to be carried by transferrin, which is a major iron transport protein in the circulation. Transferrin can bind up to two iron atoms and thus has three different states. Transferrin that is bound to two iron atom is called diferric
transferrin, one monoferric transferrin and none apotransferrin. Transferrin attaches itself to TfR1 (transferrin receptor 1) in the bone marrow to be endocytosed using clathrin-coated pits. The concentration of TfR1 is the highest in erythroid cells and varies depending on the stage that the cell is in the erythrocyte maturation process. The affinity of transferrin for TfR1 varies with the number of attached iron atoms; diferric transferrin is seven times more likely to bind to TfR1 than monoferric transferrin and a thousand times more likely than apotransferrin (Von Drygalski et al., 2012). Once transferrin is in the immature erythrocytes, they are brought to endosomes. Proton pumps on endosomes generate low pH which causes transferrin to release the iron atoms into the cytosol. An endosomal ferrireductase named Steap3 changes the oxidation state of iron once again from ferric state (Fe$^{+3}$) to ferrous (Fe$^{+2}$), and all iron atoms are brought to the mitochondria to be fixed onto heme in the last step of the heme biosynthetic pathway (Anderson et al., 2005; Von Drygalski et al., 2012)
Figure 8 – Schematic of path of dietary iron during absorption into enterocytes and transport via transferrin (Figure taken from Gisbert et al., 2003)

Regulation of iron uptake

Hepcidin is a molecule that is 25 amino acids long and is synthesized in the liver to maintain iron homeostasis. It binds to the ferroportin which is on the surface of enterocytes, and causes it to be degraded after it is endocytosed; this prevents the cells from taking up dietary iron. Hepcidin also causes the iron from the enterocytes to be exported, thus effectively trapping iron in the cells. This then leads to the decrease of serum iron concentration (Nemeth et al., 2004).

There are many factors that influence the rate of hepcidin synthesis. Hypoxia leads to an increase in erythropoiesis, and since iron is used to make hemoglobin, it will
eventually reduce hepcidin concentration. Decreased hepcidin concentrations will increase the amount of ferroportin leading to elevated dietary iron absorption (Anderson et al., 2009). High iron stores and inflammation are known to increase hepcidin concentration and iron absorption. The former is also associated with increased levels of transferrin and soluble TfR1 protein. Inflammatory signals such as interleukin (IL)-6 have been experimentally shown to increase hepcidin level in urine (Drygalski et al., 2012). Although the mechanism by which the body detects iron concentration is poorly understood, it is hypothesized that the concentration of diferric Tf may serve as an important signal (Anderson et al., 2005).

Role of iron in the body

Iron is an important element that has many functions in the body. It is a cofactor in many reactions such DNA synthesis where iron has a crucial part in reduction of ribonucleotides to their corresponding deoxyribonucleotides. It also participates in host defense and in metabolism. In the case of excess iron, which is called iron overload, highly reactive properties of the element can damage cell membranes, proteins and DNA. One of the ways to measure iron in the body is to evaluate the concentration of ferritin, which increases with excess iron in the body. The normal range of ferritin in the blood is 20-200ng/ml; at ferritin levels exceeding 1000ng/ml, one is considered to have iron overload. However, the majority of complications that can arise from the condition can be avoided at levels below 1500ng/ml. Skin is one of the most vulnerable parts of the body to ROS because there is an abundance of polyunsaturated fatty acids, which is
particularly reactive with ROS, and its constant exposure to high level of oxygen concentration and ultraviolet (UV) rays (Lambing et al., 2012).

Iron can cause damage to the body by generating reactive oxygen species, which are free radicals. Free radicals are chemical species that have an open electron shell or unpaired valence electrons. They are formed when there is enough energy and a covalent bond between two chemical species is cleaved. Balanced dissociation between them is called homolysis, and unequal cleavage is called heterolysis. Unpaired valence electrons on free radicals make it reactive, and cause them to dimerize or polymerize spontaneously.

Free radicals are generated when foreign antigens enter the body and attract macrophages. As the phagocytes process the antigens after phagocytizing the antigens, activation of NAD(P)H (nicotinamide adenine dinucleotide/nicotinamide adenine dinucleotide phosphate) oxidase cytochrome b occurs. This increases NAD(P)H while producing superoxide (\(O_2^-\)) using an oxidative burst. Oxidative burst play a crucial role in immunology as it helps the phagocytes to degrade foreign antigens. At physiological pH, superoxide that is synthesized will spontaneously lead to production of hydrogen peroxide (\(H_2O_2\)) using superoxide dismutase (SOD) as a catalyst.

\[
2O_2^- \cdot + 2H^+_2 \rightarrow H_2O_2 + O_2
\]

Free radicals can be generated when superoxide radicals (\(O_2^-\)) react with hydrogen peroxide to produce ROS.

\[
O_2^- + H_2O_2 \rightarrow O_2 + \cdot OH + OH^-
\]
Studies have shown that the two reactants of the above equation, superoxide radicals and hydrogen peroxide are not reactive in aqueous solutions and hence do not cause much damage in the body (Trenam, 1992). However, it is noted that they can generate other species that are more harmful to the body. In a process called the Fenton reaction, iron may act as a catalyst to produce ROS much more effectively than the above reaction. Free iron will react with hydrogen peroxide to yield a highly reactive hydroxyl free radical (·OH).

\[
\text{Fe}^{+3} + \text{O}_2^- \leftrightarrow \left(\text{Fe}^{+3} - \text{O}_2^-/\text{Fe}^{+2} - \text{O}_2\right) \leftrightarrow \text{Fe}^{+2} + \text{O}_2
\]

\[
\text{Fe}^{+2} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{+3} + \cdot\text{OH} + \text{OH}^-
\]

These reactions do not effectively take place in vivo, but can be accelerated in the presence of iron. Iron ion acts as a catalyst and form a perferryl species (Fe\(^{+3}\)-O\(^2\)\(^-\) or Fe\(^{+2}\)-O\(_2\)) intermediate when it reacts with superoxide radicals (O\(^2\)\(^-\)). This species then creates molecular oxygen as well as iron in ferrous state. Ferrous iron then reacts with hydrogen peroxide and forms a hydroxyl radical (Trenam, 1992; Winterbourn, 1995).

**Mechanism of Liver Fibrosis**

Studies that have investigated HCV and PCT have concluded that both diseases can lead to fibrosis in the liver (Carpintero et al., 1997). Of all patients who develop chronic hepatitis C, a quarter develops cirrhosis in 20 years, another quarter in 50 years; the remaining half does not experience in the progress in fibrosis in the liver. To date there is no HCV-induced fibrogenesis mechanism that is confirmed. It has been shown that an index that measures α₂-macroglobulin, γ-globulin, heptoglobin, γ-GT
(glutamyltranspeptidase) and bilirubin can predict the presence of liver fibrosis (Schuppan et al., 2002). A study that investigated liver biopsies of HCV positive patients who were in different stages showed that there is a relationship between down-regulation of antioxidant proteins and later stages of liver fibrosis, which reinforces the relationship between the level of liver damage and oxidative stress (Ivanov et al., 2013).

There are several hypotheses to explain the pathogenesis of liver fibrogenesis, and one of them argues that various molecules, such as collagen I, are deposited in the extracellular matrix. It is also proposed that ROS up-regulates the profibrogenic cytokine TGF-β (transforming growth factor-β) in hepatocytes and Kupffer cells in the liver (Bataller et al., 2005). This is also confirmed by other reports that concluded that elevation in cytokines in liver and plasma has a clear correlation to the TGF1β and fibrosis score (Schuppan et al., 2002). HCV proteins, such as core, NS3/4A, NS4B, NS5A, trigger MAP kinase pathways that lead to the activation of NF-κB transcription factors and TGF1β expression (Ivanov et al., 2013). Another molecule called osteopontin was discovered to play a large role in liver fibrosis as well. The molecule is induced by phosphoinositol 3 kinase, NF-κB, and phosphokinase B pathway, and leads to the increased production of collagen I and suppression of matrix metalloproteinase (MMP) 13, which alleviates scarring (Schuppan et al., 2002; Ivanov et al., 2013).

The second proposed mechanism comes from the interaction between hepatic stellate cells (HSC) and apoptotic bodies of HCV-infected hepatocytes (Ivanov et al., 2013). HSC takes up the infected hepatocytes, which leads to expression of core or E2 protein in the body, and leads to the production of TGF1β, connective tissue growth
factor (CTGF), collagen I as well as other profibrotic proteins. HCV core and E2 protein has a secondary effect where it will suppress the expression of MMP-1, which is responsible for degradation of extracellular matrix. The last mechanism of fibrogenesis is related to increased production of a particular type of proteoglycan called fibromodulin in HSC and hepatocytes. In response to oxidative stress, fibromodulin promotes proliferation and migration of HSC in the liver (Ivanov et al., 2013).

Iron Overload and Oxidative Stress In HCV and PCT

Iron and HCV infection

The direct relationship between iron and HCV infection is not very clear. However, there are many proposed mechanisms and one of them which is shown figure 9, suggests that HCV stimulates the production of HDAC (histone deactylase) and CHOP (C/EBP homology protein), both directly and indirectly using ROS. An increase in the concentration of those two molecules will lead to a decrease in C/EBP binding to the hepcidin gene promoter region which results in decreased hepcidin production. The reduction in hepcidin production leads to increased absorption of iron from the small intestine leading to overload of iron in hepatocytes (Caballes et al., 2012). This process of decreased hepcidin leading to elevated iron absorption level which then decreases hepcidin level further, and enters a cyclical process of iron overload in the liver and the body.
Figure 9 – Pathogenesis of porphyria cutanea tarda. The figure shows several risk factors, such as presence of HCV infection or increased level of ROS, which may lower the activity level of UROD in hepatocytes leading to accumulation of porphyrins in the body. HCV and ROS elevate HDAC (histone deacetylase) and CHOP (C/CBP homology protein) levels, which then inhibits the binding of CEBP (CCAAT/enhancer-binding protein) to the promoter region of hepcidin. This, along with other factors such as HFE, HJV (hemojuvelin) and TfR2 mutations, lead to the decreased expression of hepcidin and increased iron absorption in the gut. (Figure taken from Caballes et al., 2012)

There are many studies that evaluated the influence of iron on HCV replication, and it is still debatable what the direct effect is. Some studies have found that iron stimulates HCV replication (Kakizaki et al., 2000), while others have shown that iron
suppresses HCV replication. (Fillebeen et al., 2005; 2010) Still others have shown no relationship between iron concentration and the rate of HCV replication (Lehmann et al., 2010). What is clear is that HCV infection does lead to increased iron concentration in the liver and causes iron overload. HCV can cause changes in iron homeostasis which then leads to development of liver fibrosis, insulin resistance, diabetes mellitus, porphyria cutanea tarda and HCC (Negro et al., 2009). HCC mostly occurs when long-term HCV infection has caused cirrhosis in a patient (Tang et al., 2009).

**Oxidative Stress in HCV Infection**

As discussed above, iron generates hydroxyl and peroxide radicals via the Fenton reaction. The body will experience oxidative stress when the concentration of free radicals goes up and the antioxidant capacity of the cells decreases. Since iron is an important catalyst in the Fenton reaction and thus generation of ROS, it has been noted that an increase in iron probably leads to increased oxidative stress. HCV infection and replication has been shown to increase the expression of TfR1 in the liver and decrease in hepcidin levels, thus increasing iron metabolism (Caballes et al., 2012).

HCV uses several pathways to increase the concentration of ROS in the cell, and many HCV proteins are associated with the process. HCV core protein leads to the dysregulation of mitochondria, which inhibits electron transport complex I activity. This then results in increased production of ROS and increases the likelihood of apoptosis. It has also been hypothesized that an increase in expression of prohibitin, which is a chaperone protein that interacts with mitochondrial respiratory complex IV, and affects
the mitochondrial process. Core proteins are known to act directly on the mitochondrial outer membrane to dysregulate the organelle (Tang et al., 2009). Other HCV proteins also contribute to the synthesis of ROS; when HCV proteins such as E1, E2 and NS4B are expressed, they can induce stress in endoplasmic reticulum by activating antioxidant defense Nrf2/ARE (Antioxidant Response Element). This ultimately leads to increased production of hydrogen peroxide in the cells. HCV has also shown indirectly induce oxidative stress in the cell by redistributing calcium ions between the endoplasmic reticulum, cytoplasm and mitochondria. As it can be seen in figure 10, the core protein increases the activity of $\text{Ca}^{2+}$ uniporter. Core protein also inhibits SERCA and NS5A lets the calcium ions leak leading to the movement of calcium ion from endoplasmic reticulum to the cytosol. Increased concentration of intracellular calcium ion causes ROS production (Ivanov et al., 2013).

Another mechanism that HCV uses to produce ROS is through the NADPH oxidase family, which includes Nox1 – 5, DUOX1 and DUOX2. These seven NADPH oxidases are transmembrane enzymes that produce superoxide or hydrogen peroxide molecules via electron transport (Ivanov et al., 2013). Nox1 and Nox4, in particular, contribute to the production of superoxide anion and hydrogen peroxide. Nox4 is especially important because it is activated by TGFβ1 and produces those harmful species in the nucleus; oxidative species thus directly damage the DNA; and ultimately uncontrolled proliferation of the cell and cancer (Ivanov et al., 2013).
Figure 10 – The mechanism of oxidative stress that is induced in the cells infected with HCV (Figure taken from Ivanov et al., 2013)

Another way eukaryotes mediate ROS is through the Nrf2 pathway (Petri et al., 2012). Nrf2 regulates human ARE which helps the cellular expression of antioxidant enzymes such as NAD(P)H:quinoneoxidoreductase 1 (NQO1). The Nrf2 pathway is activated using several different pathways. One of the earliest discoveries showed that Keap1 (Kelch-like associated protein 1), which is a protein that is anchored to the cytoskeleton of the cell, is an important regulator of Nrf2 activity (Petri et al., 2012). During normal conditions, Nrf2 is ubiquitinated by Keap1 and consequently degraded in the proteasome. However, when there is a high concentration of electrophiles or ROS, Keap1 is inactivated and Nrf2 is stabilized (Ivanov et al., 2013). The Keap1/Nrf2 complex is regarded as an “oxidative stress sensor,” with the four cysteine residues on
Keap1 suggesting that Keap1 is the direct sensor. Oxidative stress level within the cell affects protein disulfide linkages and their rearrangement leads to the production of antioxidant chemicals (Petri et al., 2012).

![Image of the Keap1-Nrf2 system](Figure 11 - The Keap1-Nrf2 system. Under normal conditions, Nrf2 is ubiquitinated by Keap1 and degraded. When Keap1 is inactivated due to oxidative stress or electrophiles, Nrf2 accumulates in the nucleus and activates cytoprotective genes. (Figure taken from Mitsuishi et al., 2012)

Nrf2 is dissociated from Keap1/Nrf2 complex, and goes into the nucleus to initiate the transcription of genes that will produce detoxifying antioxidant proteins including NAD(P)H:quinoneoxidoreductase 1, glutathione S-transferase, and heme oxygenase-1 (Petri et al., 2012). Nrf2 targets genes that are involved in the production of glutathione, drug excretion as well as NADPH synthesis (Petri et al., 2012).
Since the discovery of Keap1, other mechanisms that depend on kinase pathways and activate Nrf2 pathway have been discovered, such as mitogen-activated protein kinases (MAPK) and phosphatidylinositol-3 kinase pathways (Hybertson et al., 2011). Although earlier studies showed that Nrf2 can only be activated after reacting with cysteine residues of Keap1, it is clear that kinase signaling mechanisms are a critical part of the activation process of Nrf2 pathway (Hybertson et al., 2011).

The Nrf2 pathway also increases glutathione synthesis. Glutathione is a major antioxidant, which is concentrated in the liver. Glutathione is a small thiol molecule that has a major function in oxidation-reduction reactions in animal cells. It is synthesized from glutamate, cysteine and glycine, which are catalyzed by two enzymes which are called glutamylcysteine synthetase and GSH synthetase. It takes up free radicals and other ROS, such as hydroxyl radical, lipid peroxyl radical, peroxynitrite and hydrogen peroxide, both directly and indirectly (Wu et al., 2004). When reacting with these species, GSH forms GSSG, which is the oxidized form of glutathione. GSSG is then reduced to give GSH using NADPH-dependent glutathione reductase (Wu et al., 2004). Patients with chronic HCV infection experience many symptoms that lead to decreased antioxidant activity in the body. First of all, it reduces the overall glutathione concentration in the body. It also increases the ratio between GSSG and GSH along with glutathione turnover.

Suppression of hepcidin is mediated by ROS, and when ROS level is increased by many mechanisms that were discussed in the above section, it can lead to lowered production of hepcidin. This causes an increase in iron metabolism and thus elevated
serum iron level. However, phlebotomy or dietary iron restriction has shown to decrease oxidative stress and lipid peroxidation in patients with chronic HCV infection (Ghany et al., 2009).

Iron and ROS in PCT

Elevated iron levels have been associated with PCT for many years, but the direct relationship between PCT and iron has not been established. Iron does not inhibit UROD activity, but is necessary for inactivation of the enzyme. In experiments performed on in vitro hepatocytes, iron facilitated the oxidation of uroporphyrinogen which can inhibit the decarboxylation process that is catalyzed by UROD (Phillips et al., 2001). Therefore, iron can be seen to cause oxidative modification in uroporphyrinogen to alter the activity level of UROD, and accelerate the inhibition process by causing the accumulation of porphyrinogens. Increased iron levels will also lead to elevation of ROS concentration in the body. The ROS creates uroporphyrin and non-porphyrin products including uroporphomethene from uroporphyrinogen. This then leads to the accumulation of uroporphyrinogen in the skin, and physical manifestation of the symptoms when it is exposed to UV rays.

Depletion of excess iron storage using phlebotomy is an effective method in managing PCT. Lowered serum iron level quickly induces the remission of cutaneous lesions and symptoms that are normally present on the skin. UROD activity level goes up gradually in patients with sPCT, and many of them improved in the next several years after phlebotomy. On the other hand, patients with fPCT did not experience an increase
in UROD activity in hepatocytes even though many of them improved clinically. Despite the fact that the relationship between PCT and iron overload is not clear, one thing that is evident is that symptoms of the disease manifest themselves at the presence of multiple risk factors.

*Relationship between Chronic HCV infection and PCT*

It is clear from many studies that there is a strong association between HCV infection and PCT. It has been demonstrated that as many as 50% of PCT patients have chronic HCV infection (Gisbert, 2003). Other studies suggest that patients with HCV infections usually develop PCT at an earlier age than others who do not have the infection. However, it is not altogether clear whether the virus itself is involved in the pathogenesis of PCT and contributes to the symptoms. It is common for patients with PCT to experience iron overload, and the removal of iron from the body prevents porphyrin overproduction and the symptoms. When iron is administered and serum iron level returns to normal, patients usually see a relapse in PCT. Since patients with PCT and HCV experience elevated iron concentration in the liver, and iron is one of the triggering factors in both diseases, it may act as one of the key factors that connect the two liver diseases.

**Conclusion**

The exact relationship between HCV infection and PCT has not been discovered, but it is clear that iron overload is one of the major factors that contribute to the development and propagation of both diseases. Iron overload leads to build up of ROS,
which then leads to increased risk of both diseases; this is especially true in the case of PCT as ROS decreases UROD activity levels. However, as research on the prevalence of the two diseases has suggested, there is no direct link between the populations of patients who suffer from HCV infection and PCT. Furthermore, studies on PCT revealed that there are many risk factors which must be present for the disease to be manifested; therefore, it is reasonable to conclude that the two diseases are not directly related. Instead, it may be that HCV infection causes PCT when other risk factors are present as well. The relationship between HCV infection and PCT will continue to be evaluated as further research identifies the causes and the mechanism behind the two diseases.
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VITA

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EDUCATION

Boston University, Division of Graduate Medical Studies, Boston, MA
Master of Arts in Medical Sciences. May 2013. GPA: 3.59/4.00

Boston College, College of Arts and Sciences, Chestnut Hill, MA
Bachelor of Arts in Theology, Pre-medical concentration. May 2011.
Honors: GPA: 3.40/4.00, Dean’s List, Theological Studies National Honor Society, Thea Bowman Scholar.

EXPERIENCE

Boston Medical Center
09/2011 – Present
Volunteer - Ophthalmology Department
- Coordinated with other members of the department to maintain and organize patient charts and medical records.

Sooowook’s Academy
Flushing, NY
Supervisor / Teacher
- Organized weekly staff meetings for 10 teachers, which included staff evaluation as well as workshops on communication and teaching skills.
- Taught mathematics, chemistry, and physics to high school students.

Mount Sinai Children’s Health Center
New York, NY
Summer 2009, 2011
- Conducted research on environmental toxins and drafted articles on them.
- Shadowed and assisted physicians in the pediatric department of Mt. Sinai Hospital.

Brigham and Women’s Hospital
Longwood, MA
09/2010 - 12/2010
Volunteer- Nutrition Department
- Verified and scheduled patient appointments for five dietitians.
- Coordinated reminder calls regarding upcoming appointments.

Options through Education- Boston College
Preceptor
Summer 2010
- Advised 40 incoming freshmen students with diverse racial and socio-economic backgrounds throughout the summer transition program.
- Facilitated dialogues in a small group setting with 5 students regarding race, religion, sexual orientation, tolerance, and student life at Boston College.
The United Saints Recovery Project/ AmeriCorps  New Orleans, LA
Summer 2009  Volunteer Staff

- Repaired homes in Garden District, New Orleans.
- Supervised a group of 30 high school volunteers in painting and drywall projects each week for seven weeks.
- Led discussions on race and education as they pertain to the history of New Orleans, LA for the student groups.

VOLUNTEER ACTIVITIES

Asian Christian Fellowship  2007 - 2011
Executive Core Member (2009-2011)

- Organized weekly large group meetings and prepared discussion topics for 35 students.
- Raised $5,000 and planned annual winter retreats, in which more than 130 students attended.
- Invited speakers to raise awareness for sex trafficking and world hunger on campus.

Katrina Relief Urban Plunge/ Habitat for Humanity  New Orleans, LA
Spring 2008, 2009, 2010

- Participated in alternative spring break to build homes with Habitat for Humanity.

AHANA Leadership Council Volunteer Corps  Mississippi Delta, MS
Winter 2011

- Assisted Teach For America corps members in classrooms during an alternative winter break trip.
- Visited high schools and local community centers to give college panels and to encourage students to pursue college education.