Influence of HCV Infection on Insulin-Like Growth Factor 1 and Proinflammatory Cytokines: Association with Risk for Growth Hormone Resistance Development

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Communications between the endocrine, immune systems and the liver have been postulated. The liver is the central organ in growth hormone/insulin-like growth factor (GH-IGF) axis. Infection with hepatitis C virus (HCV) can lead to liver problems. Although proinflammatory cytokines are an integral part of inflammation in chronic liver diseases, their involvement in mediating hepatic GH resistance during HCV infection remains to be elucidated. To address this issue, our study aimed at evaluating the influence of HCV infection on serum profile of IGF-1, TNF-α and IL-6 to assess their possible relation to hepatic dysfunction and GH resistance development. Twenty-five chronic HCV patients were studied together with 15 healthy control subjects. Serum concentration of IGF-1, TNF-α and IL-6 was determined by ELISA. HCV viral load was assessed by Real-time polymerase chain reaction using TaqMan probe technology. Basal serum GH levels were determined by a chemiluminescence assay and serum aminotransferases’ activities were also measured. TNF-α and IL-6 demonstrated higher serum levels, while IGF-1 levels were significantly lower in HCV patients compared to healthy controls. A statistically significant positive correlation was observed between GH and IL-6 levels (P<0.05), a similar trend was found between GH levels, GH/IGF-1 ratio and AST/ALT ratio (P<0.01, P<0.01, respectively). A significant negative correlation was observed between HCV viral load and GH levels (P<0.05). The progressive increase in HCV viral load matches the decrease in circulating IGF-1 levels but without reaching statistical significance. We conclude that the GH insensitivity could be induced by HCV infection and mediated by proinflammatory cytokines through their possible role in blunting the hepatic response to GH. This crosstalk between proinflammatory cytokines and GH-IGF-1 axis could be responsible for triggering impaired glucose metabolism and diabetes later on in chronic HCV infection.

Hepatitis C virus (HCV) Infection is a serious public health problem signifies an emergent challenge worldwide. Approximately 3% of the world's population has been infected with HCV, which represent about 170 million chronic carriers at risk of developing serious complications such as cirrhosis, and hepatocellular carcinoma (Drazan, 2000; Pradat & Trepo, 2000; Kato, 2001; Lavanchy, 2009).

Growth hormone (GH) is an important anabolic peptide, composed of 191 amino acids. It is expressed and released primarily from the anterior pituitary gland. GH is most well known for its regulation of carbohydrate, lipid, nitrogen and mineral metabolism (Donaghy et al., 1995; Huang et al., 2001; Woelfle et al., 2005). Many of the actions of GH are mediated by the induction of insulin-like growth factor-1 (IGF-1) expression at local sites. GH confirmed as the dominant regulator of IGF-1 gene expression and serum levels in human disease (Mathews et al., 1986). IGF-I is a polypeptide hormone with endocrine, paracrine, and autocrine effects. The primary source of circulating IGF-I is the liver. More than 90% of circulating IGF-I is synthesized in the liver (Le Roith, 1997; Conchillo et al., 2007).

The liver is the main target organ of GH in vivo and considered the central organ of the endocrine GH/IGF-1 axis (Luo et al., 2005). Liver cells have GH receptors that upon stimulation by the hormone increase IGF-I gene transcription; once synthesized, IGF-I is
released into plasma. IGF-I inhibits GH secretion both directly, by acting on the pituitary, and indirectly, by stimulating somatostatin secretion in the hypothalamus, which in turn inhibits GH release (Jones & Clemmons, 1995; Blomsma et al., 1997; Conchillo et al., 2007). Thus a negative feedback circuit is established. Acquired GH resistance, characterized by low concentrations of IGF-I with respect to normal or elevated GH levels (Picardi et al., 2006) (Fig. 1).

Close relationships between the endocrine system and the liver, also, between the immune and endocrine systems have been proposed. The IGF system is an attractive target in this respect since it may be modified by liver diseases and, reciprocally, it might play a relevant role in the progression of some hepatic diseases. An evolving theme over the past 20 years is the idea that GH and IGF-I play a role in the development, maintenance and function of the immune system (Kelley et al., 2007).

Interleukin 6 (IL-6) and tumor necrosis factor (TNF)-α are powerful proinflammatory cytokine with pleiotropic properties. They are among the main mediators of the antiviral inflammatory response in HCV infection (Oyanagi et al., 1999; Cotler et al., 2001; Liu & Han, 2001; Falasca et al., 2006). Although proinflammatory cytokines are an integral part of inflammation in chronic liver diseases (CLD), their involvement in the pathogenesis of GH resistance during HCV infection remains controversial.

The present study was designed to investigate the influence of HCV infection on serum profile of IGF-1, TNF-α and IL-6 and assess their potential relationship with hepatic dysfunction and GH resistance development.

![Figure 1. Circulating GH-IGF-1 Axis in Health and GH Resistance Syndrome. (as modified from Le Roith D. Insulin-like Growth Factors. N Eng J Med 1997; 336: 633-40).](image-url)
Subjects and Methods

A group of 25 chronic HCV patients (eighteen males and seven females) aged between 35 and 60 years, attending the Medical Research Institute together with 15 sex- and age- matched healthy control subjects were included in the present work. All the subjects met the inclusion criteria that for the patient group were absence of: history for gastrointestinal system bleeding; suspicion of hepatocellular carcinoma or any other malignancy; diabetes mellitus; and alcohol intake and for the control group were absence of: diabetes mellitus and other chronic illnesses; history of malignancy; impaired liver function tests; and alcohol intake.

The diagnosis of HCV infection was based on abnormal serum aminotransferases levels for > 6 months’ duration, positive testing for anti-HCV antibodies by ELISA (Abbott-Murex, Anti-HCV, Version 4.0, Murex Biotech Limited. Central Road. Dartford, UK) and positive HCV RNA by polymerase chain reaction (PCR). None of these patients received antiviral treatment. Blood samples from all subjects were tested for hepatitis B surface antigen (HBsAg) to exclude HBV infection by ELISA (Abbott-Murex, HBsAg, Version 3.0, Murex Biotech Limited. Central Road. Dartford, UK).

HCV-RNA viral load was measured by means of a Real-time quantitative PCR technique. HCV RNA was extracted from 140µl of serum using QIAamp® viral RNA mini kit [Qiagen GmbH, Germany], according to manufacturer's instructions. HCV viremia was analyzed and quantified by Real-time PCR [Mx3000PTM Real-Time PCR System, Stratagene] using TaqMan probe technology [Roche- Applied biosystems]. TaqMan reactions were performed in 25 µl reaction volume. All reactions were carried out in MicroAmp optical tubes sealed with MicroAmp optical caps. HCV RNA was amplified according to the following program: 1 cycle each of 48ºC for 30 min and 95ºC for 10 min, followed by 40 cycles each of 95ºC for 15 sec and 60 ºC for 1 min.

Basal (level without stimulation) serum GH level was determined by chemiluminescence assay according to manufacturer's instructions [IMMULITE®/IMMULITE® 1000 Growth Hormone (hGH) chemiluminescence assay kit]. It is a solid-phase, chemiluminescent immunometric assay. The solid phase (bead) is coated with murine monoclonal anti-hGH antibody. The reagent contains alkaline phosphatase conjugated to a rabbit anti-hGH polyclonal antibody. The reagent and hGH in the sample are incubated together with a bead coated with a murine anti-hGH monoclonal antibody to form an antibody sandwich complex. Unbound enzyme conjugate is then removed by a centrifugal wash. Finally, chemiluminescent substrate is added to the bead and signal is generated in proportion to the bound enzyme. Serum IGF-1 concentrations were evaluated using commercial ELISA kits [DRG®IGF-1(Insulin-like Growth factor), DRG Instruments GmbH, Germany], according to manufacturer's instructions. The sensitivity of serum GH and IGF-1 assay is 0.01 ng/ml and 0.15 ng/ml, respectively. Immunological parameters [TNF-α and IL-6] were determined by Enzyme Immunoassay method with Bender MedSystems kits (BMS223/4 and BMS213/2 Bender MedSystems GmbH, Vienna, Austria; Europe; respectively), according to kit prescription. Serum aminotransferases [ALT and AST] activities were measured with an automated analyzer (Open system, ChemWell® autoanalyzer, U.S.A.)

The AST/ALT ratio has been used to noninvasively assess the severity of disease in patients with chronic liver disease since it reflects progressive liver functional impairment and fibrosis. It is considered one of the best predictors of fibrosis progression in chronic hepatitis C (Giannini et al., 2001; Ghany et al., 2003). The GH/IGF-1 concentration ratio was estimated to get a better understanding of the relative changes in concentration of GH, IGF-1 for better estimation of GH resistance (Ichikawa et al., 2007).

The study was approved by the Ethical Committee of the Medical Research Institute, University of Alexandria and informed consent was obtained from the study subjects. All samples were tested under code.

Statistical Analysis

Statistical analyses were carried out with the Statistical Program for Social Sciences [SPSS version 14]. All data were expressed as means ± SEM (Standard Error of mean). Correlation between data was analyzed with Pearson’s correlation coefficients. Comparisons between two groups were performed using Student’s t test. Differences were considered statistically significant when P<0.05.

Results

As compared with the controls, HCV infected subjects demonstrated higher TNF-α and IL-6 serum levels. For HCV infected cases, the mean TNF-α serum concentration was 52.19±18.87 pg/ml, whereas TNF-α serum
concentration were below the detection limit (2.3 pg/ml) of the assay in the healthy control group (Fig. 2). IL-6 demonstrated a significantly higher mean serum level in HCV infected group as compared to healthy subjects (8.25±4.99 pg/ml vs. 1.30±0.76 pg/ml, \(P=0.001\)) (Fig. 3). On the other hand, IGF-1 levels were significantly lower in HCV patients as compared to the controls (146.53±17.48ng/ml vs. 225.00±20.14ng/ml, \(P<0.05\)) (Fig. 4).

In HCV patients, concentrations of serum GH correlated positively with IL-6 levels (\(P<0.05\)). In contrast, no correlation between serum GH levels and TNF-α was found. A significant positive correlation was observed between GH levels and AST/ALT ratio (\(P<0.01\)), a similar trend was found between GH/IGF-1 ratio and AST/ALT ratio (\(P<0.01\)). Furthermore, in the HCV patients, the progressive increase in HCV viral load matches the decrease in circulating IGF-1 levels but without reaching statistical significance, while, a significant negative correlation was observed between HCV viral load and GH levels (\(P<0.05\)) (Table 1).

**Figure 2.** Comparison between HCV Patients and Controls Regarding Mean Values (pg/ml) of TNF-α.

* TNF-α serum concentrations were below the detection limit of the assay.
Figure 3. Comparison between HCV Patients and Controls Regarding Mean Values (pg/ml) of IL-6.

Figure 4. Comparison between HCV Patients and Controls Regarding Mean Values (ng/ml) of IGF-1
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Table 1. Relationship between Growth Hormone, Growth Hormone-to-Insulin-like Growth Factor-1 Ratio and HCV RNA Viral Load, Interleukin-6 Levels and Aspartate Aminotransferase-to-Alanine Aminotransferase Ratio.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>r</th>
<th>p</th>
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<tbody>
<tr>
<td>GH and HCV RNA viral load</td>
<td>-0.400</td>
<td>0.047</td>
</tr>
<tr>
<td>GH and IL-6</td>
<td>0.451</td>
<td>0.024</td>
</tr>
<tr>
<td>GH and AST/ALT ratio</td>
<td>0.526</td>
<td>0.007</td>
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<tr>
<td>GH/IGF-1 ratio and HCV RNA viral load</td>
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</tr>
<tr>
<td>GH/IGF-1 ratio and IL-6</td>
<td>0.356</td>
<td>0.081</td>
</tr>
<tr>
<td>GH/IGF-1 ratio and AST/ALT ratio</td>
<td>0.515</td>
<td>0.008</td>
</tr>
</tbody>
</table>

GH= Growth Hormone; IGF-1= Insulin-like Growth Factor-1; IL-6= Interleukin 6
AST= Aspartate aminotransferase ALT=alanine aminotransferase.

Discussion

HCV induces inflammatory signals leading to chronic hepatitis and its consequences. TNF-α and IL-6 were among the main proinflammatory cytokines released in response to hepatocellular stress associated with hepatitis C viral infection (Kasprzak et al., 2004). In the present study, we demonstrated that HCV patients have significantly higher levels of TNF-α and IL-6 than healthy controls. These findings are in agreement with previous reports (Huang et al., 1999; Mammaev et al., 2001; Neuman et al., 2001; Kasprzak et al., 2004; Lecube et al., 2006). Increased levels of TNF-α and IL6 may have some role in hepatic injury including the inflammatory process of chronic hepatitis C. The major cell type for TNF-α production in the liver is Kupffer cells which release TNF-α when activated by factors released by damaged hepatocytes (Ramadori & Armbrust, 2001; Prosser et al., 2006). However, the role of TNF-α and IL-6 in the pathophysiology of chronic viral hepatitis is not fully understood, moreover; the cells responsible for IL-6 production in the diseased liver remains to be elucidated.

Oyanagi et al., (1999) reported that activated hepatic stellate cells may produce and secrete IL-6 by the stimuli of TNF-α which are derived from activated Kupffer cells. This IL-6 overproduction could be integrated in the inflammatory response system, which is activated during HCV infection.

In view of the fact that the liver is the main source of circulating IGF-1; and its production is under the control of GH. IGF-1 may be considered a marker of functional reserve or hepatocellular functional capacity. Reduced IGF-1 levels are likely to eventually result from two factors: a decrease in GH receptors seen in cirrhotic livers (Chang et al., 1990; Shen et al., 1998; Donaghy et al., 2002) and a progressive reduction of liver synthesis capability from decreased hepatocellular mass in advanced stages. Therefore, reduced IGF-1 levels could reflect hepatocellular dysfunction and severity of liver impairment (Colakoğlu et al., 2007; Conchillo et al., 2007). In agreement with these data, the present work shows a significantly lowered IGF-1 serum levels in HCV patients than controls. In addition, a significant positive correlation was observed
between GH levels and AST/ALT ratio also between GH/IGF-1 ratio and AST/ALT ratio.

Similar to our results, a significant reduction in hepatic production of IGF-1 has been reported previously (Morali et al., 2005) in patients with chronic liver disease. In addition, our findings are in accordance with Del Monte et al., (1995) who reported an increased IGF-1 concentration during therapy that may reflect an improvement in patients with hepatic disease. In the present work, serum IGF-1 concentrations were decreased in spite of normal or increased GH; this means that the liver becomes unresponsive to GH with consequent development of GH resistance. Increased GH could be accounted for by a lack of negative feedback on its secretion when plasma IGF-1 declines.

The concept of important physiological interaction between the endocrine and immune system has been assumed as they share a common biochemical language involving shared ligand and receptors including neuroendocrine hormones, growth factors and cytokines (Kelley et al., 2007). Picardi et al., (2003) reported that proinflammatory cytokines play an important role in the pathogenesis of GH resistance in CLD. This could initiate new therapeutic lines of attack in the management of CLD.

A role for proinflammatory cytokines in modulating the GH–IGF-1 axis and induce a state of GH resistance has been hypothesized (Yumet et al., 2002). Since expression of proinflammatory cytokines could mediate the necroinflammatory changes in the diseased liver, this assumes the possible role of proinflammatory cytokines in blunting the hepatic response to GH. In our work, basal concentrations of serum GH in HCV patients correlated positively with IL-6 levels.

IL-6 could act as a potential mediator of hepatic-GH resistance in patients with HCV positive-hepatitis. Both IL-6 and GH, belonging to the cytokine receptor superfamily and can transduce their signal from cell surface to nucleus through the same pathway (Kishimoto et al., 1995; Heinrich et al., 1998). Hence, the negative feedback to IL-6 biological activities that happened as a result of elevated IL-6 levels induced by HCV infection may also inhibited the GH intracellular signal transduction. Another way is that IL-6 could inhibit GH-mediated gene expression in hepatocytes (Ahmed et al., 2007).

On the contrary; in the present work, there was no correlation between basal serum GH and TNF-α levels. However, a previous study suggested that GH could modulate TNF-α release (Bozzola et al., 1998). Conversely, a stimulatory effect of TNF-α on the endocrine system has been postulated (Elsasser et al., 1991) as TNF-α is capable of modulating pituitary secretion by interacting directly with the endocrine system, in particular with pituitary cells. However, another suggestion reported by Ahmed et al., (2006) that TNF-α inhibits growth hormone-mediated gene expression in hepatocytes.

In the present study, no correlation was observed between HCV viral load and either TNF-α or IL-6 which is consistent with Frese et al., (2003) who suggests that the increased production of TNF-α seen in many hepatitis C patients does not contribute to HCV clearance by inducing antiviral defense mechanisms in infected hepatocytes. In contrast, Falasca et al., (2006) reported in HCV positive group of patients that, IL-6 correlates positively with the viral load.

Interestingly, in the present work, the progressive increase in HCV viral load matches the decrease in circulating IGF-1 levels, with a significant negative correlation between HCV viral load and GH levels. This could reflect progressive reduction in hepatocellular mass with disease progression. Therefore, GH resistance worsens in parallel with the progression of liver disease.

We conclude that the GH insensitivity could be induced by HCV infection and
mediated by proinflammatory cytokines through their possible role in blunting the hepatic response to GH since the way of communication between the immune and endocrine systems is activated when the inflammatory processes induced by proinflammatory cytokines antagonize the function of GH, which then causes GH resistance. Therefore, this cross-talk between proinflammatory cytokines and GH-IGF-1 axis could be responsible for triggering impaired glucose metabolism and diabetes later on in chronic HCV infection. This finding provides further argument emphasizing the importance of understanding the molecular details of communication systems between the immune and endocrine systems.

References


