Correlation and Multiple Regression Analyses of Pituitary Growth Hormone and Hepatic Activities in Hepatitis C Infection and Interferon Response

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Received: 3 December 2012 / Accepted: 5 February 2013 / Published online: 16 February 2013
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Abstract The prevalence of hepatitis C virus (HCV) infection varies across the world, with the highest percent of infections reported in Middle East, increasingly in Egypt. The current study aimed at examining the bio-statistical correlation and multiple regression analyses of pituitary growth hormone (GH) and liver activities among HCV genotype-4 patients treated with PEG-IFN- α plus RBV therapy. Herein, the current study was conducted on 100 HCV genotype-4 infected patients and 50 healthy controls. Patients received PEG-IFN- α /RBV for 24 weeks. Host RNA was isolated from patients' sera for HCV genotyping and viral load determination. Moreover, the enzymatic activities of the liver, AFP, GH, PT, and CBC were performed in all volunteers. The present study resulted that the activities of

the hepatic enzymes among HCV genotype-4 patients correlated together significantly. While, human GH showed a significant positive regression with pre-treatment ALT concentration in responders. Furthermore, multiple regression analysis for GH showed a significant positive correlation with pre-treatment ALT in HCV genotype-4 infected patients. We concluded that there were a putative significant relation between GH and pre-treatment ALT activity in HCV infection and response to IFN-based therapy.

Keywords Hepatitis C · Interferon · Pituitary growth hormone · Liver enzymes

Introduction

Positive single stranded RNA viruses require the extensive use of host cell functions to perform their replication cycles in mammalian cells. Moreover, host cell functions are required for several steps in their intracellular replication cycles, including viral translation and RNA synthesis [1]. Hepatitis C virus, which belongs to the genus *Hepacivirus* of the family *Flaviviridae*, is a single stranded, positive sense RNA virus with a genome sequence of approximately 9,500 nucleotide [2].

The prevalence of hepatitis C virus (HCV) infection varies across the world [3], with the highest number of infections reported in Middle East, increasingly in Egypt [4, 5]. Most chronic infections lead to hepatitis and to some degree of fibrosis and develop liver cirrhosis. At this stage of the disease the risk of developing hepatocellular carcinoma, the third leading cause of cancer-related death worldwide, is 1–4 % per year [6]. Surgical, local ablative, chemotherapy options are currently available for HCC treatment. Recently, there has been a growing interest in

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the therapeutic use of natural products for liver carcinoma [7], because systemic chemotherapy has been unsuccessful in the studies thus far [8].

In 1998, a first randomized clinical trial testing a dual-therapy with interferon- α 2b (IFN- α 2b) and ribavirin (RBV), an oral administration of the broad-spectrum antiviral, increased sustained virological response (SVR) rates considerably to 35–43 % and replaced mono-therapy [9]. More than 3 years later, pegylated IFN- α (PEG-IFN- α), IFN- α tethered with a polyethylene glycol (PEG), with a much longer serum half-life was introduced [10]. Finally, the addition of ribavirin to PEG-IFN- α significantly ameliorates the outcome of chronic hepatitis C therapy [11, 12].

During the first 3 months of therapy, HCV viral loads usually fall to undetectable levels in response to interferon. However, in non-responders (NR) patients HCV viral loads persist as the pretreatment levels. Among those patients with an initial response to treatment up to 50 % will relapse after treatment is discontinued and known as relapsed responders (RR), whereas the remainder will have a sustained viral response (SVR) as determined by the absence of detectable viraemia 6 months after treatment has stopped [13–15].

Intriguingly, the most powerful on-treatment predictors are the viral kinetics on treatment [16] and the most important pre-treatment predictor so far is the viral genotype; mainly because genotypes 4 and 1 patients are poor responders to IFN therapy compared to genotypes 2 or 3 patients [17]. Another very important factor which is powerfully involved as a pre-treatment predictor is the host gene. Various genetic polymorphisms of IL28B [18, 19], 2'-5'-OAS [20], and BCL2 [21–23] were examined previously with the HCV response to the IFN-based therapy. These genetic polymorphisms provided good examples of pre-therapeutic host gene predictors for interferon response.

There were several factors which are associated with HCV infection and are known to be contributed with disease prognosis and response to treatment. These factors are gender, age, source of infection, liver transaminases (ALT and AST), α -fetoprotein (AFP), HCV-RNA level (viral load), viral genotype, grade of activity and fibrosis. Recently, it was found that non-responders have higher viral load and higher grade of liver activity and fibrosis than in responders but with no significant difference [21]. Conversely, several studies have shown that high viral load and high grade of liver activity and fibrosis are negatively related with SVR to interferon treatment [24].

The frequency of the liver transaminases (ALT, AST) and the tumor marker (AFP) was elevated within the infected patients with hepatitis C type-4 [25–29]. Moreover, it was demonstrated that AFP was negatively associated with treatment response in HCV patients [30]. There are high significant differences of post-treatment ALT and AST

on response to treatment. Furthermore, early normalization of an abnormal ALT level may indicate response to antiviral treatment [31, 32].

Recently, scientists suggested that growth hormone (GH) insufficiency is common in patients infected with chronic hepatitis C virus. They added that basal and stimulated growth hormone concentrations increased significantly during antiviral therapy [33–35]. Some of them added that there was high significant increase of GH in responders compared to non-responders after treatment [35].

Overall, all previous findings encouraged us to establish this study which examined the bio-statistical correlation and multiple regression analyses of the human pituitary growth hormone and liver enzyme levels as well as AFP and viral RNA load within hepatitis C infection in addition to the response to PEG-IFN- α and RBV combination therapy.

Materials and Methods

Patients Criteria

The current study was conducted on 150 volunteers and divided into two major groups; 100 HCV genotype-4 infected patients group and 50 healthy controls group. HCV patients visiting the clinics of the Tropical Medicine and Hepatology Department at El-Kasr El-Aini Hospital were enrolled in the prospective study after signing the consent form. HCV patients received PEG-IFN- α /RBV for 24 weeks.

HCV-RNA was examined at week 24. Patients who tested undetectable RNA; continued treatment until week 48 to detect sustained virological response (SVR) when the viral RNA remains undetected after 6 months of cessation of treatment. While those were tested detectable RNA; stopped treatment at week 24 and were classified as non-responders (NR). The study protocol and informed consent were approved by the Ethics Committee of Cairo University and National Research Centre.

HCV patients participated in the present study fulfilled the following inclusion criteria: age 20–50 years, elevated ALT and AST (>37 IU/L); within 6 months prior to entry the study, hemoglobin (>11 g/dL), total white blood cells ($>3,000/\text{mm}^3$), neutrophil ($>1,500/\text{mm}^3$), platelets ($>100,000/\text{mm}^3$), prothrombin time within normal range (9.8–13.8 s), positive HCV antibodies and RNA, HCV-RNA type-4, no liver cirrhosis, no hepatocellular carcinoma (HCC), negative hepatitis B surface antigen (HBsAg), negative human immunodeficiency virus (HIV) and negative active schistosomiasis.

Hepatitis Viruses Detection

Antibodies and antigens of hepatitis C virus (HCV), hepatitis B virus (HBV) and hepatitis immunodeficiency

virus (HIV) were detected using standard viral tests. HCV patients who have liver-associated viruses other than hepatitis C virus were excluded from the prospective study.

Regarding to hepatitis C virus determination, real time reverse transcription polymerase chain reaction (qRT-PCR) was performed for all patients to confirm the seropositively results obtained from standard viral tests. RNA for viral load determination was extracted from sera using a Qiagen Viral RNA kit (Hilden, Germany). HCV-RNA quantification was performed by qRT-PCR using a LightCycler system (Roche Diagnostics GmbH, Mannheim, Germany). HCV-RNA in serum was measured before treatment and routinely at weeks 24 and 48 after treatment and graded into low, moderate and high levels.

Hepatitis C Genotype-4 Determination

RNA was isolated from sera for HCV genotyping using a Qiagen Viral RNA kit (Hilden, Germany). HCV-RNA genotyping was performed using Ohno method which depends on nested PCR amplification of the virus core gene using genotype specific primers mentioned previously [36].

Determination of Biochemical Activities

The enzymatic activities of aspartate amino transferase (AST) and alanine amino transferase (ALT) were determined using kits provided by Pointe Scientific Company USA, according to the method described by Tietz [37]. Moreover, prothrombin time (PT), alpha fetoprotein (AFP), complete blood picture (CBC), and GH were performed in hepatitis C genotype-4 infected patients and healthy controls.

Histopathological Investigations

Chronic hepatitis C infected patients were subjected to abdominal ultrasound and liver biopsy was taken from each patient before the onset of the therapy to estimate the grade of activity and fibrosis according to metavir scoring system [38].

Bio-statistical Analyses

The data were bio-statistically analyzed using computer program: Statistical Package for the Social Science (SPSS; SPSS Inc., Chicago, version 17.0, USA). A probability value (P value) <0.05 was considered statistically significant. Moreover, the current study aimed at investigating the ratio of the covariance between liver transaminases (ALT and AST) as well as AFP and viral RNA quantity to the variance of the GH as predictor variable (regression) or the ratio of the covariance to the square root of the product the variances

(correlation). Furthermore, predicting a single variable from the weighted linear sum of multiple variables (multiple regression) or measuring the strength of this relationship (multiple correlation) was statistically performed.

Results

Parameters Correlation

Tables 1 and 2 illustrate different correlations between the measured virological, clinical, and biochemical parameters including; age, ALT, AST, AFP and viral load among HCV genotype-4 patients prior and post IFN-based therapy. Table 1 showed that all measured parameters have no significant differences ($P > 0.05$) when correlated each parameter individually with other ones in pre-treatment HCV genotype-4 infected patients. While Table 2 illustrated that there were high significant differences when correlated AST with ALT/AFP, ALT with AST/AFP, and AFP with ALT/AST in post-treatment HCV genotype-4 infected patients. Conversely, there were no significant differences when correlate age or viral load with ALT, AST and AFP in post-treatment HCV genotype-4 patients (Table 2).

Regression Analyses

Tables 3, 4 and Figs. 1, 2, 3, 4, 5, 6, and 7 summarize the different regressions between the human GH and the different measured clinical and biochemical parameters including; age, ALT, AST, and AFP in chronic HCV genotype-4 diseased group, healthy control group, responder group, and non-responder group. GH showed a non-significant negative regression with age in responder subjects ($r = -0.374$, $P = 0.055$) as illustrated in (Table 4). Also, GH showed a significant positive regression with pre-treatment ALT concentration in responder subjects ($r = 0.435$, $P = 0.023$) as illustrated in both (Table 4) and (Fig. 1a).

Table 1 Correlations between age, ALT, AST, AFP and viral load among HCV patients genotype-4 before treatment

Parameters (P value)	Age	AST	ALT	AFP	Viral load (qRT-PCR)
Age	–	0.291	0.433	0.500	0.385
AST	0.351	–	0.322	0.169	0.662
ALT	0.433	0.322	–	0.776	0.860
AFP	0.300	0.269	0.776	–	0.435
Viral load (qRT-PCR)	0.275	0.662	0.760	0.435	–

All data were represented in term of P value

$P > 0.05$ non-significant difference

Table 2 Correlations between age, ALT, AST, AFP and viral load among HCV patients genotype-4 after treatment

Parameters (<i>P</i> value)	Age	AST	ALT	AFP	Viral load (qRT-PCR)
Age	–	0.945	0.147	0.855	0.106
AST	0.945	–	0.001**	0.028*	0.564
ALT	0.147	0.001**	–	0.011*	0.697
AFP	0.855	0.028*	0.011*	–	0.258
Viral load (qRT-PCR)	0.106	0.564	0.697	0.258	–

Bold numbers represent statistically significant changes

All data were represented in term of *P* value

P > 0.05 non-significant difference

* *P* > 0.05 significant difference

** *P* > 0.01 high significant difference

Table 3 Regressions between ln growth hormone and measured parameters in pre-/post-treatment HCV genotype-4 patients and healthy controls

Parameters (Ln-transformed)	HCV patients		Healthy controls	
	<i>r</i>	<i>P</i> value	<i>r</i>	<i>P</i> value
Age	–0.091	0.519	–0.085	0.603
ALT, “A”	0.161	0.254	–0.034	0.835
ALT, “B”	0.112	0.428	–	–
AST, “A”	–0.070	0.624	0.001	0.996
AST, “B”	0.131	0.356	–	–
AFP, “A”	–0.152	0.284	0.054	0.742
AFP, “B”	0.080	0.574	–	–

r correlation coefficient, A before IFN/RBV therapy or pre-treatment, B after IFN/RBV therapy or post-treatment

Furthermore, regression analysis for GH showed that all measured clinical and biochemical parameters were not independent predictors of GH in healthy controls, patients and non-responder subjects as illustrated in both (Table 3) and (Figs. 1b–7).

Multiple Regression Analyses

Tables 5, 6, 7, and 8 summarize the different multiple regressions between the human GH and the different measured clinical and biochemical parameters including; age, ALT, AST, and AFP in chronic HCV genotype-4 diseased group, healthy control group, responder group, and non-responder group. Multiple regression analysis for GH showed a significant positive correlation with pre-treatment ALT in HCV genotype-4 infected patients (*P* = 0.038) as shown in (Table 6). Furthermore, multiple regression analysis for GH showed that all measured clinical and biochemical parameters were not independent predictors of growth hormone in healthy controls, responders and non-responder subjects as illustrated in (Tables 5, 7, 8).

Table 4 Regressions between ln growth hormone and measured parameters in pre-/post-treatment responder and non-responder patients

Parameters (Ln-transformed)	Responders		Non-responders	
	<i>r</i>	<i>P</i> value	<i>r</i>	<i>P</i> value
Age	–0.374	0.055	0.189	0.365
ALT, “A”	0.435	0.023*	–0.006	0.976
ALT, “B”	0.120	0.552	0.205	0.326
AST, “A”	–0.178	0.375	0.015	0.943
AST, “B”	–0.019	0.927	0.360	0.077
AFP, “A”	–0.096	0.635	0.081	0.701
AFP, “B”	0.034	0.867	0.200	0.338

r correlation coefficient, A before IFN/RBV therapy or pre-treatment, B after IFN/RBV therapy or post-treatment

* Significant difference

Discussion

The current findings showed that post-treatment AST, ALT and AFP in infected patients with hepatitis C genotype-4 correlated significantly with ALT and AFP, AST and AFP, and ALT and AST respectively. Moreover, pituitary GH showed a significant positive correlation with pre-treatment ALT concentration in responders. Furthermore, multiple regression analysis for GH showed a significant positive correlation with pre-treatment ALT in HCV genotype-4 infected patients.

In the present results, there are no correlations between growth hormone and age, pre- or post-treatment liver transaminases (ALT, and AST), and AFP in HCV genotype-4 infected patient, pre- and post-treatment groups as well as controls. This finding was supported by authors who found that transaminases have not correlated with growth hormone concentrations before or during therapy [33]. However, our observation found a significant positive correlation between growth hormone and pre-treatment

Fig. 1 **a** Ln GH and Ln pre-treatment ALT in responders. *Asterisk* represent high statistically significant difference. **b** Ln GH and Ln post-treatment ALT in responders

Fig. 2 **a** Ln GH and Ln pre-treatment AST in responders, **b** Ln GH and Ln post-treatment AST in responders

Fig. 3 **a** Ln GH and Ln pre-treatment AFP in responders, **b** Ln GH and Ln post-treatment AFP in responders

ALT concentration in responder subjects and infected patients.

The current hypothesis may be clarified by the following mechanism of action illustrated the potential correlation between different enzymatic liver activities including; ALT,

AST and insulin-like growth factor-1 (IGF-1) in addition to GH and AFP among HCV infected patients treated with PEG-IFN- α and RBV combination therapy (Fig. 8). Firstly, human brain hypothalamus releases growth hormone releasing hormone (GHRH) which induces the pituitary

Fig. 4 **a** Ln GH and Ln age in non-responders. **b** Ln GH and Ln pre-treatment ALT in non-responders

Fig. 5 **a** Ln GH and Ln post-treatment ALT in non-responders. **b** Ln GH and Ln pre-treatment AST in non-responders

Fig. 6 **a** Ln GH and Ln post-treatment AST in non-responders. **b** Ln GH and Ln pre-treatment AFP in non-responders

gland to activates some immune cells including; macrophages, B-cells, T-cells, and natural killer cells to secrete GH. Furthermore, GH stimulates the liver cells, through the

JAK-STAT signaling pathway, to produce IGF-1. It was deduced that the liver is a major target organ of GH for this process and is the principal site of IGF-1 production [39–41].

Fig. 7 Ln GH and Ln post-treatment AFP in non-responders

Additionally, the HCV infection led to GH and IGF-1 levels to be decreased through two feedback inhibition pathways; the first one is to inhibits the GH production from the pituitary gland directly and the second indirect way is to activates the hypothalamus to releases somatostatin which in turn, inhibits the pituitary gland to releases more GH.

Over 25 years ago, the first clinical trials with recombinant human IFN- α have been conducted and IFN- α has remained the backbone of HCV therapies [42]. IFN- α has

no direct interaction with HCV but rather indirect antiviral and immunomodulatory effects. IFN- α induces interferon stimulated genes (ISGs), through the Jak-STAT pathway, that lead to a non-virus-specific cellular antiviral state [43]. Sustained virological responders treated with IFN will have renormalized GH, IGF-1, ALT, AST levels, while AFP remains within normal range.

The ribavirin addition to pegylated interferon (IFN) significantly ameliorates the outcome of chronic hepatitis C therapy [11]. Authors showed that ribavirin's principal effect is to maintain the response to pegylated IFN so that a response is achieved [12]. Many hypotheses have been raised to explain ribavirin's action in the treatment of hepatitis C. They include: (i) mechanisms that could explain an antiviral effect of ribavirin, such as inhibition of inosine monophosphate dehydrogenase (IMPDH), that would deplete intracellular GTP pools; a moderate direct inhibitory effect on HCV RNA-dependent RNA polymerase [44]. Moreover, mutagenic properties that would lead to 'error catastrophe', i.e., the generation of non-viable HCV quasiespecies [45]; and (ii) mechanisms that could explain an immune modulatory effect of ribavirin, such as a switch toward T-helper 1 responses, leading to a more efficient elimination of infected cells by specific immune effectors during IFN- α therapy [44].

Table 5 Multiple regression analysis for ln growth hormone in Healthy controls

Independent variable	Healthy controls			
	B-regression coefficient	SE of regression coefficient	t value	P value
Intercept	2.104	4.202	0.501	0.620
Ln age	−0.343	0.934	−0.411	0.683
Ln ALT, "A"	−0.109	0.465	−0.235	0.815
Ln ALT, "B"	—	—	—	—
Ln AST, "A"	−1.535E−02	0.798	−0.019	0.985
Ln AST, "B"	—	—	—	—
Ln AFP, "A"	−7.949E−02	0.616	−0.129	0.898
Ln AFP, "B"	—	—	—	—

SE standard error, A before IFN/RBV therapy or pre-treatment, B after IFN/RBV therapy or post-treatment

Table 6 Multiple regression analysis for ln growth hormone in HCV genotype-4 patients

Independent variable	HCV patients			
	B-regression coefficient	SE of regression coefficient	t value	P value
Intercept	−1.045	2.550	−0.410	0.684
Ln age	−0.341	0.626	−0.545	0.589
Ln ALT, "A"	0.737	0.344	2.143	0.038*
Ln ALT, "B"	0.106	0.338	0.314	0.755
Ln AST, "A"	−0.501	0.437	−1.147	0.258
Ln AST, "B"	0.147	0.356	0.413	0.681
Ln AFP, "A"	−1.139	0.672	−1.695	0.097
Ln AFP, "B"	0.895	0.472	1.895	0.065

SE standard error, A before IFN/RBV therapy or pre-treatment, B after IFN/RBV therapy or post-treatment

* Significant difference

Table 7 Multiple regression analysis for ln growth hormone in responder patients

Independent variable	Responders			
	B-regression coefficient	SE of regression coefficient	<i>t</i> value	<i>P</i> value
Intercept	0.685	4.005	0.171	0.866
Ln age	−1.083	0.731	−1.481	0.155
Ln ALT, “A”	1.125	0.543	2.072	0.052
Ln ALT, “B”	0.743	0.742	1.001	0.329
Ln AST, “A”	−0.872	0.477	−1.827	0.083
Ln AST, “B”	−0.384	0.651	−0.590	0.562
Ln AFP, “A”	−0.652	1.024	−0.637	0.532
Ln AFP, “B”	0.857	0.857	1.124	0.275

SE standard error, *A* before IFN/RBV therapy or pre-treatment, *B* after IFN/RBV therapy or post-treatment

Table 8 Multiple regression analysis for ln growth hormone in non-responder patients

Independent variable	Non-responders			
	B-regression coefficient	SE of regression coefficient	<i>t</i> value	<i>P</i> value
Intercept	−3.379	4.806	−0.703	0.491
Ln age	0.198	1.260	0.157	0.877
Ln ALT, “A”	0.116	0.689	0.169	0.868
Ln ALT, “B”	9.999E−03	0.492	0.020	0.984
Ln AST, “A”	−0.244	1.005	−0.242	0.811
Ln AST, “B”	0.569	0.573	0.993	0.334
Ln AFP, “A”	−0.940	1.499	−0.627	0.539
Ln AFP, “B”	0.929	1.112	0.835	0.415

SE standard error, *A* before IFN/RBV therapy or pre-treatment, *B* after IFN/RBV therapy or post-treatment

Fig. 8 Schematic diagram illustrates the mechanisms correlate GH and liver enzymes as well as AFP during HCV infection and after IFN therapy

Finally, we concluded that HCV infection and the IFN-response have a putative significant impact on the positive relationship between GH and pre-treatment ALT activity. While there were no effect on the enzymatic activities of the liver.

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