RESEARCH PAPER | Science | Volume : 5 | Issue : 2 | Feb 2015 | ISSN - 2249-555X

Biochemical Studies on Serum Adeponectin Level as Predictor to Interferon Alpha Therapy in Egyptian Chronic Hepatitis C Patients.

KEYWORDS: liver, Hepatitis C Virus, Interferon Alpha, Adeponectin.

Mohamed S. Elghareeb
Chemistry Department, Faculty of science, Port Said University, Port said, Egypt.

Gamil M. Abd-Allah
Biochemistry Department, Faculty of Pharmacy (Boys), Al-AzharUniversity, Cairo, Egypt.

Lamis G.Ashour
Chemistry Department, Faculty of science, Port Said University, Port said, Egypt.

ABSTRACT *Aim of study: To investigate serum level of Adeponectin compared with response of interferon (IFN)-α treatment; in three stages among Egyptian patients with chronic hepatitis C virus infection.*

Subjects and Methods: This study was performed on 30 subjects admitted to endemic diseases unit of the Cairo Fatimic Hospital (CFH), from which 20 patients suffering from chronic hepatitis C and 10 healthy volunteers as normal control group. Levels of Adeponectin, AST, ALT, Total Bilirubin, Creatinine, HCV RNA and CBC were determined in sera and blood samples of individuals at the time of admission (before beginning of treatment), four weeks and twelve weeks later (after beginning of treatment). Adeponectin was determined using enzyme-linked immunosorbent assay (ELISA) method. AST, ALT, T.Bilirubin and Creatinine were determined spectophotometrically and HCV RNA was determined by polymerase chain reaction assay (PCR) method.

Results: Adeponectin's serum level at three stages of treatment were high by significant in week zero (P=0.0053) and decreased significantly in both week four (P=0.0001) and week twelve (P=0.0375), while serum level of the following parameters AST, ALT, Total Bilirubin and Creatinine showed a gradual decrease with treatment time (P >0.1). Regarding three groups of treatment, serum HCV RNA show significance of difference between three samples (P 0.08 in W0, 0.2 in W4 and > 0.1 in W12).

Conclusions: This study concluded that, HCV may directly affect adiponectin. This is further supported by the significant increase in adiponectin at the end of treatment. Serum adiponectin at baseline appears to be an independent predictor of liver steatosis and for the achievement of the end of treatment virological response

Introduction:

The liver is reddish brown organ with four lobes; it is located in the upper right quadrant of abdominal cavity [1]. Liver have multiple function as protein synthesis, lipid metabolism and toxic substances break down. Defect in one of physiological functions of liver, leads to liver diseases [2]. One of the most common is HCV which is a chronic infection can lead to scarring and cirrhosis [3]. Cytokines are abroad and loose category of small protein that are released by cells and affect the behavior of other cells that include chemokines, adepokines, interferons, interleukins,lymphokines, tumor factors. The adepokines are cytokines secreted by adipose tissue for ex: adeponectin, leptin, apelin…etc. Interferon alpha that interfered with viral replication for ex: HCV treatment [4]. The pathogenesis of steatosis in patients with HCV is not well un-derstood [5]. Moreover, HCV-related steatosis is not always virally related and other factors may coexist. Obesity is a well-recognized risk factor for the development of steatosis and of fibrosis in HCV-infected patients [6]. Adipose tissue has traditionally been considered an energy storage organ, but over the last decade, a new role has emerged for the adipose tissue as an endocrine organ ·Adipose tissues secrete a variety of hormones including adiponectin, and leptin which may contribute to the development of metabolic abnormalities [7].

Adiponectin is an adipocytokine secreted by adipocytes, with antidiabetic, antilipogenic and antiatherogenic actions.Two adiponectin receptors, AdipoR1 and AdipoR2, have been cloned, several types, including macrophages, express adiponectin receptors. These have distinct tissue specificities within the body and have different affinities to the various forms of adiponectin. The receptors affect the downstream target AMP kinase, an important cellular metabolic rate control point [8]. Expression of the receptors is correlated with insulin levels, as well as reduced in diabetes persons, particularly in skeletal muscle and adipose tissue. Liver expresses both receptor genes and has the highest expression of AdipoR2 among the organs [3]. Low plasma levels of adiponectin are associated with increased insulin resistance and with an altered lipid pattern. Adiponectin levels correlate negatively with liver fat and hepatic insulin resistance in diabetic patients and in their healthy relatives. Similarly, low serum adiponectin levels have been reported in non-alcoholic fatty liver disease, where they were associated with increased transaminase levels and worse histology. Adiponectin directly affects the inflammatory response by regulating both the production and activity of cytokines and can also act as an antiapoptotic agent in a variety of cell types. In both alcoholic and non-alcoholic fatty liver, adiponectin administration suppresses hepatic production and the circulating levels of tumour necrosis factor (TNF)-$^{\alpha}$ and ameliorates hepatic steatosis. The anti-inflammatory effects of adiponectin could protect the liver from the development of inflammation and cell injury [9].

All the above led to the concept that adiponectin as antilipogenic and anti-inflammatory factor might be related to features of HCV chronic hepatitis. We hypothesized that in patients with chronic hepatitis C, the anti-inflammatory actions of adiponectin may have a role in ameliorating disease severity [10].

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Subjects and methods:
Study population: This study included 30 persons; 20 patients were chronic HCV with mean age 34.6 ± 1.74 years, they were (40%) female and (60%) male recruited from attendants endemic diseases unit at Cairo Fatimic hospital, 10 healthy volunteers as normal control group (6 males and 4 females), their mean age were 30.2 ± 2.0 years. The studied HCV patients were divided according to adeponectin level into three stages; 20 (33 %) samples (Group 1) in week 0 before treatment (interferon injection), with adeponectin level higher than 50 ng/ml , 20 (33%) samples (Group 2) in week 4 after interferon treatment with adeponectin level between 20-50 ng/ml and 20 (33%) samples (Group 3) that in week 12 after interferon treatment with adeponectin level lower than 20 ng/ml.

Sampling:
Ten ml venous blood samples were collected from patients and divided into 8 ml clean sterile tubes and 2ml on EDTA containing tubes. Serum was separated and subdivided into three aliquots. The first aliquot was used for analysis of creatinine, AST, ALT, and Total bilirubin. The second aliquot was used for analysis of adeponectin, HCV RNA. The third aliquot was used for blood analysis of Hb and white blood cells.

Laboratory investigations were performed including; serum adeponectin concentation was measured with ELISA using (Boster immunoleader) kit according to the method of [11]. Total Bilirubin, AST, ALT [12] and Creatinine was measured with automated method by using SYNHROR CX4 PRO (Beckman) according to the method of [13]; Serum HCV RNA was measured with using QIAGEN® OneStep RT-PCR Kit.

Results:
The collected data were tabulated and statistically analyzed using GraphPad InStat Version 3 for windows XP and Graph Pad Prism 5 (GraphPad Software, Inc.). All data were expressed as mean ±SEM. The level of statistical significance was taken at P<0.05, comparison of variables among groups of the study was made by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test to judge the deference between various groups.

Table 1: General parameters of thecontrol (C) group and all HCV groups (W0=Group2), (W4=Group3) and (W12=Group4) in all characters (Mean±SEM).

Characters	Group(1)(C) N=10	Group (2),(3),(4) N=20	P
Age (year)	26.2±3. 4	37.3±4.9	0.1
Weight (kg)	75.5±5.2	83.22±2.5	0.1
Height (cm)	176.8±1.1	175.1±1.3	0.4
Systolic B.P. (mmHg)	121.3±1.8	135.6±1.7	< 0.0001
Diastolic B.P. (mmHg)	82.5±0.8	89.8±1.2	0.0005

Table 2: Comparison between serum prameters of control (C) group and all HCV treatment stages groups in all parameters (Mean±SEM).
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Parameters	Control n=10	Group (1) n=20	Group (2) n=20	Group (3) n=20	P
Adeponectin (ng/ml)	3.9±0.5	7.9±1.2	5.8±0.9	3.9±0.6	0.001
Wbc (count*103)	7.0±0.5	7.1±0.4	5.8±0.3	3.8±0.4	0.0001
Hb %	13.4±0.5	14.1±0.4	13.1±0.4	10.8±0.3	0.002
AST (IU/L)	22.3±2.9	45.5±4.4	29.3±2.6	20.9±1.5	0.001
ALT (IU/L)	22.2±3.3	57.8±6.2	33.2±3.2	22.7±1.9	0.0001
T.Bilirubin (mg/dl)	0.4±0.1	0.8±0.1	0.7±0.1	0.7±0.1	0.0001
Creatinine (mg/dl)	0.8±0.1	0.8±0.1	0.9±0.1	0.9±0.1	0.001
HCV RNA (IU/ml)	50± 0	23546±546	6450±635	351±127	0.08

Table 3: Comparison between week 0 before treatment group and week 4, week 12 stages after treatment groups in all parameters (Mean±SEM).

Parameters	W0 Group (1) n=20	W 4 Group (2) n=20	W12 Group (3) n=20	P
Adeponectin (ng/ml)	17.6±1.0	11.0±0.9	4.2±0.3	0.002
Wbc (count*10^3)	5.9±0.3	4.8±0.2	2.8±0.1	0.0001
Hb%	14.1±0.7	11.8±0.2	9.6±0.6	0.001
AST (IU/L)	57.0±4.9	28.0±2.9	16.2±1.8	0.0002
ALT (IU/L)	74.8±4.9	34.5±2.4	17.0±2.1	0.0002
T.Bilirubin (mg/dl)	0.7±0.1	0.7±0.1	1.13±0.1	0.03
Creatinine (mg/dl)	0.6±0.1	0.8±0.1	1.1±0.1	0.01
HCV RNA (IU/ml)	23546±546	8175±835	162±122.5	0.001

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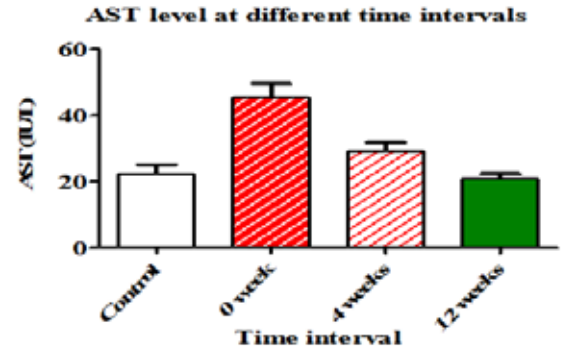

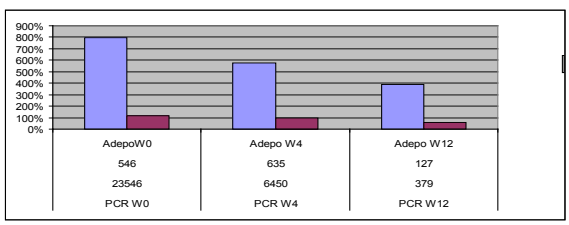

Figure (1) : Relation between Adeponectin and HCV RNA

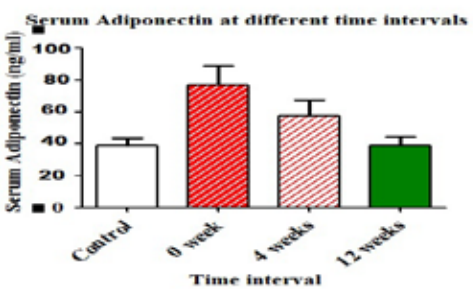

Figure (2) : The (Mean±SEM) serum level of Adeponectin in control and three stages of treatment.

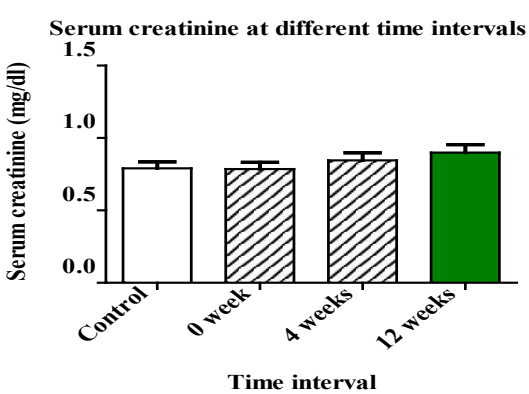

Figure (3) : The (Mean±SEM) serum level of creatinine in control and three stages of treatment.

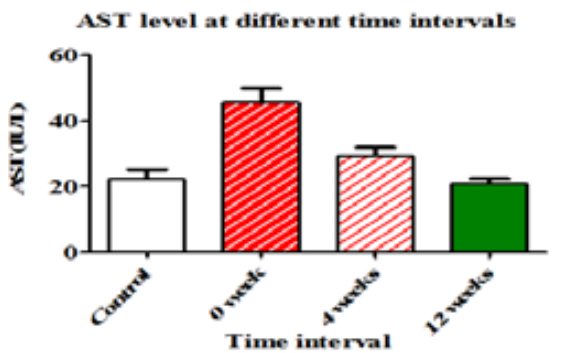

Figure (4) : The (Mean±SEM) serum level of AST in control and three stages of treatment.

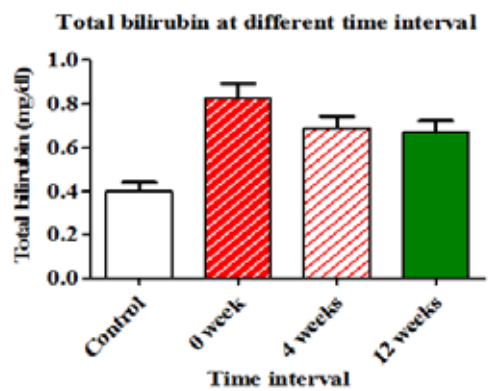

Figure (5) : The (Mean±SEM) serum level of T.Bilirubin in control and three stages of treatment.

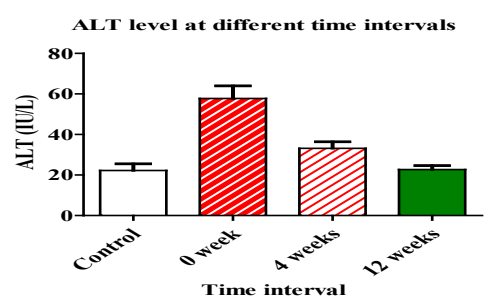

Figure (6) : The (Mean±SEM) serum level of ALT in control and three stages of treatment.

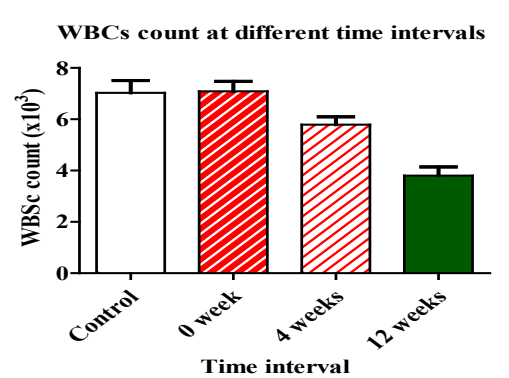

Figure (7) : The (Mean±SEM) serum level of Wbc in control and three stages of treatment.

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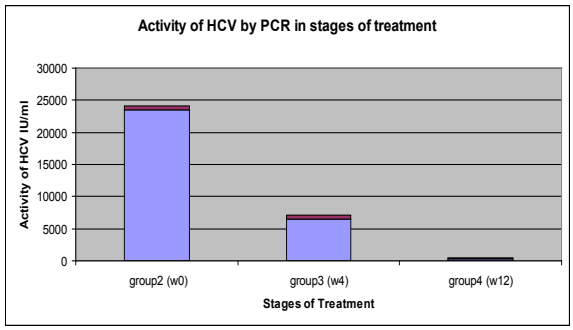

Figure (8) : The (Mean±SEM) serum level of HCV RNA in three stages of treatment.

Discussion

The first important finding of this study is that adiponectin values of patients with chronic HCV infection were heigh. It has been reported that adiponectin is generally elevated in patients with chronic liver disease than in healthy controls. HCV-infected patients presented more liver inflammation (higher AST, ALT and higher grading score). This difference in liver inflammation can be attributed to the higher percentage of previous antiviral treatment and the high response rate to therapy in the HCV group. It can be postulated, that in patients with chronic hepatitis C, that the increased serum adiponectin in relation to hepatic inflammatory activity may be secondary to the response to viral infection.

Two hypotheses may demonstrate why plasma adeponectin was elevated with in week 0 before CHC patient beginning treatment. The hypothesis of stress represents the first one, where plasma adeponectin level raised directly, affects the inflammatory response by regulating both the production and activity of cytokines and can also act as an antiapoptotic agent in a variety of cell types. In both alcoholic and non-alcoholic fatty liver, adiponectin administration suppresses hepatic production and the circulating levels of tumour necrosis factor (TNF)-α and ameliorates hepatic steatosis. The anti-inflammatory effects of adiponectin could protect the liver from the development of inflammation and cell injury [(6)]. The second hypothesis is the visceral adiposity is associated with elevated circulating free fatty acids [(14)].

The decline in plasma adeponectin in week four and twelve after treatment mean decreased inflammation, where adeponectin can also act as an anti inflammatory agent. Low plasma levels of adiponectin are associated with increased insulin resistance and with an altered lipid pattern. Adiponectin levels correlate negatively with liver fat and hepatic insulin resistance in diabetic patients and in their healthy relatives. Similarly, low serum adiponectin levels have been reported in non-alcoholic fatty liver disease, where they were associated with increased transaminase levels and worse histology [(3)].

Our study revealed that ALT, AST, T.Bilirubin, Creatinine and ALP are highly significantly higher in HCV group in three stages of treatment than controls, there is significant correlation found between adiponectin and liver enzymes in the HCV studied group. That liver function and Adeponectin is high in week 0 and slightly decreased in w4 and more decreased in w12 that during injection of interferon among 12 week that refer to decrease liver inflammation subsequently patient response to treatment. An association between plasma adiponectin and liver functions was found also in healthy subjects. Lopeza A. and Botas P.(2004)[(15)] reported that adiponectin levels were significantly correlated with ALT, AST and T.Bilirubin independently of sex, age and BMI suggesting a wider role for adiponectin in the maintenance of liver integrity.

Analysis of variance Creatinine indicated significant difference between the mean values of concentration between the two groups control and patients groups Week four and week twelve**.** Guebre ., et al.(2005)**[(16)]** also found that decrease in adiponectin with increasing renal function. Serum adiponectin showed an inverse association with BMI.

With the continuation of our studied we noticed that there is a significance between control and diseased groups in CBC. Aso Y, et al . (2006)[(17)] stated that the presence of anemia may contribute to the elevated serum levels of total adiponectin.

On the other hand, we noticed that a positive relationship between adeponectin and HCV RNA that when patient response to treatment inflammation will decreased otherwise adeponectin decreased and HCV count will decreased among the end of treatment as our study concluded. Contrary to our previous report **Zografos et al (2006)[(18)]** where leptin alterations were attributed to the direct effect of IFN-α, the significant increase in adiponectin in the present study among HCV treated patients is most likely due to viral depletion and/or a decrease in the viral load and not to a single direct effect of IFN-α.

In conclusion, the important finding of our study is that adiponectin values of patients with chronic HCV infection were high. It has been reported that adiponectin is generally elevated in patients with chronic liver disease than in healthy controls. HCV-infected patients presented more liver inflammation (higher AST, ALT and higher grading score). This difference in liver inflammation can be attributed to the higher percentage of previous antiviral treatment and the high response rate to therapy in the HCV group. It can be postulated, that in patients with chronic hepatitis C, that the increased serum adiponectin in relation to hepatic inflammatory activity may be secondary to the response to viral infection.

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