MONOCYTE CHEMOTACTIC PROTEIN AND RESPONSE TO PEGYLATED INTERFERON-ALPHA-2A TREATMENT IN PATIENTS WITH CHRONIC HEPATITIS C (CHC) GENOTYPE 4

By

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Abstract

The prevalence of hepatitis C virus (HCV) infection varies across the world, with the highest number of infections reported in Egypt. Monocyte chemotactic protein-1 (MCP-1) is a potent chemokine, and its hepatic expression is up-regulated during chronic HCV infection. Fifty naive patients with chronic hepatitis C in National Hepatology & Tropical Medicine Research Institute and 20 healthy volunteers as controls were enrolled in a prospective study designed with strict inclusion criteria to nullify the effect of confounding variables and further minimize selection bias. Fifty naïve patients were treated with PEG-IFN-a2b, at a dose of 180lg/kg subcutaneously every week plus ribavirin at a dose of 1000–1200 mg/day, according to the patient's body weight, for 48 weeks. Quantification of HCV-RNA by real-time PCR and MCP-1 by ELISA were performed for every patient and controls. There was a statistically significant difference between patients and control group as regards the quantity of MCP-1 (P <0.05) (Mann–Whitney test) (P =0.004). There was a significant difference between responders and nonresponses regarding MCP-1 (P < 0.05), responders showed a higher percentage of cases with initial MCP-1< 306 (P < 0.05). We conclude the importance of the detection of MCP-1 expression at the start of therapy as a factor for assessing the likelihood of HCV genotype 4 patients to achieving a sustained virological response to treatment with IFN-a2 in combination with ribavirin.

Keywords: Egypt, Genotype 4, Hepatitis C virus, Interferon, MCP-1.

Introduction

Hepatitis C virus (HCV) related liver disease is a common and clinically significant condition (Batts et al, 1995). It is considered to be one of the most common chronic viral infections worldwide (Shaker et al, 2010). The prevalence of HCV infection varies across the world, with the highest infections reported in Egypt (Frank et al, 2000; Kamel et al, 2014). HCV infection affects 2-3% of the population, approximately 170 million people worldwide, causing chronic HCV-related hepatitis with subsequent liver cirrhosis, hepatic failure, hepatocellular cancer, and liver-related mortality in a large number of patients (Hunyady et al, 2011). Interferon α (IFN-α) was the only agent available for the treatment of CHC patients. Addition of ribavarin (RBV) to IFN-α increased the SVR rate to about 50% (Fried et al, 2002). Pegylated IFN-α inert polyethylene glycol (Peg) attached to conventional IFN-α, was introduced into clinical practice, and based on the longer half-life of this drug form. Peg-IFNα markedly improved SVR rates and offered the further advantage of weekly rather than thrice a week administration. Interestingly, the SVR rates obtained with Peg-IFNα plus RBV were significantly higher in patients infected with HCV gt-2 or gt-3 (76-82%) than in those infected with gt-1 or gt-4, 42-46% (Poynard et al, 1998). Over the last decade, the standard of care for chronic hepatitis C treatment is the combination of pegylated-interferon-alfa (PEG-IFN) & ribavirin which resulted in sustained virological response (SVR) rates of 75%-85% in patients with genotypes 2 or 3 but only of

40%-50% in patients with the genotype 1 (Alexopoulou et al, 2012). Peginterferon-alpha-2a (40 kD) is a new 'pegylated' subcutaneous formulation of interferon-alpha-2a that has been developed to improve on the pharmacokinetic profile and therapeutic efficacy of interferon-alpha-2a. Peginterferon-alpha-2a (40 kD) is produced by covalent attachment of recombinant interferon-alpha-2a to a branched mobile 40 kD polyethylene glycol moiety, which shields the interferon-alpha-2a molecule from enzymatic degradation, reduces systemic clearance and enables once-weekly administration (Perry et al, 2001). The host immune response plays an important role in viral clearance in patients who are chronically infected with hepatitis C virus (HCV) and are treated with interferon and ribavirin. Activation of the immune system involves the release of pro and anti-inflammatory molecules measured in plasma samples (Moura et al, 2011). Wald et al. (2007) mark chemokines and their receptors as key players in leukocyte recirculation via inflamed liver. Monocyte chemoattractant protein-1 (MCP-1/CCL2) is one of the key chemokines that regulate migration and infiltration of monocytes /macrophages. Both CCL2 and its receptor CCR2 demonstrated to be induced and involved in many diseases. Migration of monocytes from blood stream across vascular endothelium is required for the routine immunological surveillance of tissues and in response to inflammation (Deshmane et al, 2009). The monocyte chemotactic protein-1 (MCP-1) is a potent chemokine, and its hepatic expression is up-regulated during chronic HCV infection mainly in activated hepatic stellate cells (HSC) (Mühlbauer et al, 2003). Studies reported that MCP-1 concentration may be a prognostic marker of efficacy of IFN+RBV therapy in chronic hepatitis C patients. In chronic hepatitis C before and during treatment, serum levels of MCP-1 in responders were similar to healthy subjects. In non-responders (NR), MCP-1 increased in the course of IFNalpha+RBV treatment, differ-

ences were significant as compared to responders. MCP-1 correlated statistically with periportal inflammation activity but without liver fibrosis staging (Panasiuk et al, 2004). Pretreatment MCP-1 levels in patients with sustained virological response (SVR) were significantly lower than in non-responders (Non-SVR) & MCP-1 significantly decrease in patients with SVR but not in Non-SVR 48 weeks post-treatment of (Gu et al, 2014).

Subjects and Methods

In this prospective study, sample size was 70 subjects divided into two groups. GI: consists of 20 healthy volunteers with cross-matched age and sex, men-to-women ratio was 12/8, with age ranged from 18 to 58 years. GII: consists of 50 naïve patients to be treated with PEG-IFN-a2b, at a dose of 180lg/kg subcutaneously every week plus ribavirin at a dose of 1000-1200 mg/day, according to patient's body weight, for 48 weeks, with men-to-women ratio of 34/16; their ages ranged from 20 to 54 years. Strict inclusion criteria were set to nullify the effect of confounding variables and minimize selection bias. Inclusion criteria were adult men or women (18-60 years) with proven chronic hepatitis C genotype 4, elevation of aspartate aminotransferase and alanine aminotransferase levels, positive serum HCV-RNA by quantitative PCR, chronic hepatitis evidence in liver biopsy, Hb≥13 gm/dl in males and 12gm/dl in females, albumin> 3.5 gm, naive patients (not previously treated with any antiviral drugs including IFN, ribavirin, thymosin and lamivudine). Exclusion criteria were decompensated liver disease, histological evidence of hepatic cirrhosis proved by hepatic histopathology, pregnant or nursing female, concomitant the hepatic schistosomiasis (excluded by rectal snip and pathologically), alcohol intake, other etiologies of chronic hepatitis (e.g. autoimmune, HB virus infection and drug-induced liver injury) and presence of any chronic systemic illness, pre-existing ischemic cardiovascular disease, antiviral or the immunosuppressive therapy within last 6 months, Severe pre-

existing psychiatric conditions, Patients with thyroid disease or if patient had an abnormal baseline TSH level, Uncontrolled D.M, Abnormal fundus examination, anemia (Hb<13gm/dl in males & 12gm/dl in females), Chronic renal disease, all subjects included were subjected to the following:

Medical History: Full history was taken with special reference to risk factors for liver diseases such as previous HCV exposure in surgical wards, blood transfusions, dental therapy, and needle stick injury, history of HCV in the spouse and i.v. injection.

Physical examination: Complete medical examination with particular focus upon the manifestations of hepatitis such as jaundice, hepatomegaly, and tenderness in the right hypochondrium. BMI was calculated as body weight in kgm divided by square of height in meters (kg/m^2). Abdominal ultrasonography was performed for all patients.

Laboratory investigations: Morning venous blood samples were taken after 12-h overnight fast. Plasma HbA1c, complete blood count (WBC's, Hb), Platelet count (Plt), Prothrombin Time (PT) measurement (normal PT was 12 seconds). Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), albumin (Alb), direct and total bilirubin levels (Bil), glucose and Creatnine were measured by standard clinical laboratory methods by Beckman CX4. TSH and a-fetoprotein levels were estimated by serologic techniques for all patients.

Viral markers: ELISA assays: Sera of patients and control were tested for HBsAg, TSH and a-fetoprotein before treatment by using ELISA. All patients were tested for MCP-1 before and after treatment by ELISA.

Quantitation of HCV-RNA in serum: HCV-RNA was quantitated in all patients' serum samples using Real Time PCR (RT-PCR). Low viremia was defined as viral load less than 100 x10^3 IU/L, moderate viremia as viral load 100-1000×10^3 IU/ L, and high viremia when viral load >1000 x10^3 IU/ L.

Statistical analysis: All data were revised for completeness and accuracy, pre coded data was entered on the computer using the statistical package of social science software program, version 15(SPSS) to be statistically analyzed, data was summarized using (Mean and SD for quantitative variables- Number and percent for qualitative variable), comparison between quantitative variables were done using independent T test for quantitative variable which where normally distributed and nonparametric Mann-Whitney tests for quantitative variables which were not normally distributed and Wilcoxon test used for comparison of two related quantitative variables, ROC (receiver operator characteristic curve) was used to find out the best cut off and validity of certain variable, P-value less than 0.05 was consider of significant.

Results

Baseline characteristics: The age of the control group ranged from 18–58 years, patients ranged from 20-54 years. Sex distribution in GI was 8 (40%) females and 12 (60%) males, in GII females were 16 (32%) and number of males was 34(68%). There was no significant difference between the three groups as regards the distribution of age and sex. Correlation between the sex and MCP-1 level showed that there was no significant difference between male and female regarding MCP-1 level (Fig 1). Comparison between patients before and after treatment showed significantly higher mean value of biochemical factors (AST, ALT, HB & WBC) before treatment while after treatment group showed significantly higher PLT count and MCP-1 values, the mean serum MCP-1 level in the patient group was 279.6±86.5 ng/ml before initiation the PEG-IFN therapy. After 48 weeks of PEG- IFN therapy, the mean serum level elevated to 343.6 ± 77.2 ng/ml.

Table 1: Baseline characteristics of 50 patients infected with HCV Genotype 4.

Mean ±SD	Before treatment	After Treatment	P value
AST	62.6±24.2	46.7±23.9	0.003 (S)
ALT	59.2±19.6	36.3±10.5	<0.001(S)
T.BIL.	1.1±0.4	3.0±8.1	0.101
D.BIL.	0.3±0.2	0.3±0.3	0.379
GLU.	99.2±17.3	96.5±30.9	0.606
AFP	13.0±12.9	15.1±22.5	0.572
Hb	12.0±1.8	10.0±1.7	<0.001(S)
TSH	3.7±0.8	3.7±1.5	.953 (NS)
Creatinin	1.0±0.1	1.1±0.3	.020 (S)
PLT	291080.0±74451.9	98012.4±103871.4	<0.001(S)
BMI	27.0±9.4	24.4±7.7	.110(NS)
MCP1	279.6±86.5	343.6±77.2	<0.001(S)
WBC	8024.0±1395.1	5206.4±1742.9	<0.001(S)

Table 2: Correlation between responders and non-responders regarding MCP-1 level.

	No.	Mean± SD of MCP1	median(iqr)	P value
Non responders	18	405.3±82.7	405.0(317.3:426.3)	<0.001
Responders	32	308.9±47.1	290.0(281.5:331.3)	(S)

Correlation between responders and non-responders regarding MCP-1 level. There was a significant correlation between MCP-1 level and response to treatment (P<0.001).

Level of MCP-1 was higher in non-responders group than in responders group represented as median (405, 290) respectively.

Table 3: Comparison between responders and non responders regarding all parameters.

	Non responders (N=18)	Responders (N=32)	P value
AST	59.2±21.6	64.5±25.7	.584
ALT	61.6±15.7	57.8±21.6	.235
T.BIL.	1.1±0.5	1.1±0.4	.911
D.BIL.	0.3±0.2	0.3±0.2	.883
ALb.	3.9±0.4	3.8±0.3	.217
GLU.	95.4±15.5	101.4±18.0	.130
AFP	11.4±7.1	14.0±15.2	.670
Hb	12.7±1.7	11.6±1.8	.043 (S)
PCR	$4170x10^6 \pm 17428x10^6$	$1250x10^6 \pm 5487x10^6$	.189
TSH	3.8±0.4	3.6±0.9	.361
Creatinine	0.9±0.1	1.0±0.2	.059
PLTs	$318x10^3 \pm 82x10^3$	$275x10^3 \pm 66x10^3$	.048(S)
BMI	26.1±8.6	27.5±10.0	.418
MCP1	318.4±91.4	257.7±76.7	.028 (S)
WBC	8044.4±1350.0	8012.5±1441.0	.943
Fibrosis	3.0±1.1	3.3±0.7	.433

AST: Aspartate transaminase, ALT: Alanine transaminase, T.BIL: total bilirubin, D.BIL: direct bilirubin, ALB: albumin, GLU: glucose, AFP: alpha-fetoprotein, Hb: hemoglobin, PCR: Polymerase Chain Reaction, TSH: Thyroid Stimulating Hormone, PLT: platelets, BMI: Body Mass Index, MCP-1: Monocyte Chemoattractant Protein 1, WBC: White Blood Cells.

Comparison between responders and non-responders to laboratory parameters showed that non responders group was significantly higher in mean value of Hb, PLT & MCP-1.

The highest sensitivity (88.9%) at MCP-1 level 306 and highest specificity (71.9) for prediction the response to treatment showed (Tab. 4, Fig 3).

Table 4: Cut off value of MCP-1 for prediction response to treatment

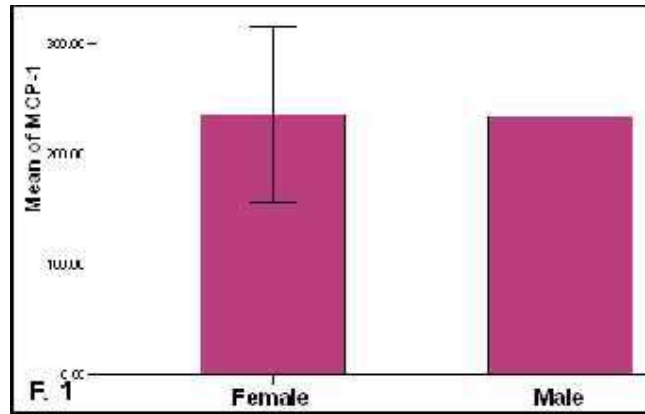| Cut off	Sensitivity	Specificity
306.0	88.9	71.9

Fig 1: Correlation between MCP-1 and sex (MCP-1: Monocyte chemotactic Protein 1)
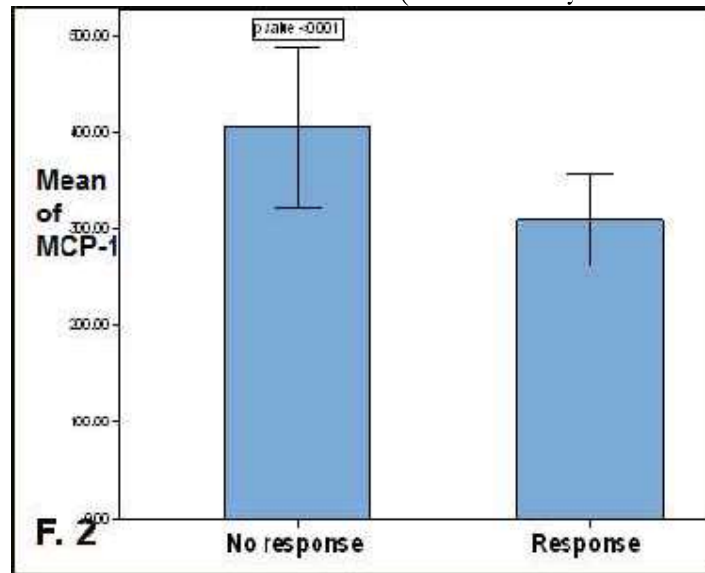

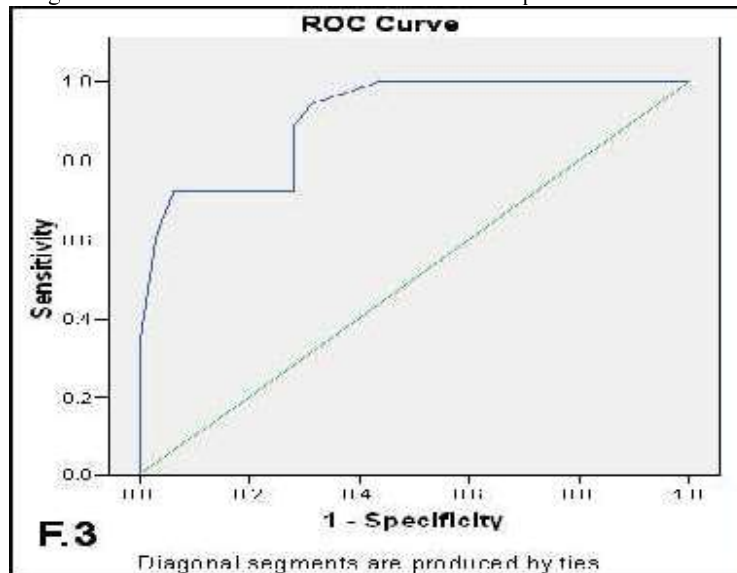Fig. 2: Correlation between MCP-1 level and response to treatment.

Fig. 3: ROC curve of MCP-1 for prediction of response.

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Discussion

Chemokines is a subgroup of small cytokines involved with leukocyte trafficking through a process called haptotaxis where leukocytes move towards higher concentrations of chemokines. This process is important for the regulation of leukocyte migration into sites of infection or into lymph nodes (Ono *et al*, 2003). Chemo-attraction of CTL to the liver seems essential to HCV eradication. As chemokines regulate the movement of leukocytes throughout the body they seem to play a key role in treatment response (Shields *et al*, 1999). Monocyte chemotactic protein-1 (MCP-1/CCL2) is a β-chemokine that has been suggested to be responsible for monocyte and lymphocyte T recruitment in acute inflammatory conditions and may be an important mediator in chronic inflammation.

In fact, it has been proposed that CCL2 is responsible for tissue inflammation in autoimmune diseases, as documented with tissue expression in human and experimental autoimmune animal models (Asano *et al*, 2000). Chronic liver diseases are associated with increased hepatic and monocytic expression of monocyte chemotactic protein 1 (MCP-1). MCP-1 level was higher in hepatic veins than in peripheral blood and occurred in severe cases of liver disease (Fisher *et al*, 1999).

The hepatic expression of MCP-1 is up-regulated during the chronic HCV infection mainly in activated hepatic stellate cells (HSC). In chronic hepatitis C with advanced fibrosis and inflammation, hepatic MCP-1 mRNA levels were significantly higher (Muhlbauer *et al*, 2003). Peripheral blood monocytes and activated HSC are the source of MCP-1. Monocyte chemotactic protein-1 recruits monocytes & lymphocytes to damag area in liver tissue. Profibrinogenic properties of MCP-1 could be reflected by induction of HSC chemotaxis and transformation to myofibroblasts (Marra *et al*, 1999).

Several independent studies highlighted the importance of chemokine receptor CCR2 and its cognate ligand monocyte-chemoattractant protein 1 (MCP-1/CCL2) for monocyte recruitment during experimental hepatic fibrosis (Imamura *et al*, 2005). Panasiuk *et al*. (2004) found that MCP-1 concentration may be a prognostic marker of the efficacy of IFN+RBV therapy in patients with chronic hepatitis C. They showed that MCP-1 concentrations before and during the treatment in non-responders were higher than those in responders and increased during treatment and this agreed with the present results. Moura *et al*. (2011) found that no association between plasma levels of the CC chemokines evaluated (CCL2) and virological response and this disagree with the present results. The study showed that HCV sustained virological response after PEG-INF therapy was associated with MCP-1 serum level. High MCP-1 levels predispose patients failed to therapy respond. Cut off value of MCP-1 equal 306 above which patient might not respond to treatment. There was significant relation between the MCP-1 level and AST, ALT & PCR of HCV), but without significant relation between MCP-1 level before & after treatment and fibrosis (P>0.01). Florholmen *et al*. (2010) found that MCP-1 increased at 24 hours treatments in sustained response group and in relapser one, but not in non-responder group which disagreed with the present data.

The present results proved that MCP-1 was a chemokine reflecting the inflammatory activity in liver and a prognostic factor of treatment efficacy with IFN alpha and RBV. Patients with a persisting long-lasting response to treatment revealed MCP-1 concentrations statistically lower and during treatment it did not undergo significant changes. Patients with negative effect of the therapy showed higher MCP-1 levels and increased significantly during the IFN+RBV treatment. Moreover, the present study showed the possible role for the serum MCP-1 level in predicting the antiviral therapy outcome in the HCV genotype 4 patients.

Conclusion

High level of MCP-1 predicts an unfavorable response to antiviral treatment of HCV. The results highlighted the importance of detection of MCP-1 level at start of therapy as a predictable factor for assessing the likelihood of HCV genotype 4 SVR for IFN-a2 treatment in combination with ribavirin.

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